EVOLUTION

INTERNATIONAL JOURNAL OF ORGANIC EVOLUTION

PUBLISHED BY

THE SOCIETY FOR THE STUDY OF EVOLUTION

Vol. III **SEPTEMBER, 1949** **No. 3**

THERMOREGULATION IN REPTILES, A FACTOR IN EVOLUTION

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Received December 27, 1948

INTRODUCTION

Vertebrates are commonly divided into two groups, the "cold-blooded" or poikilothermic, and the "warm-blooded" or homoiothermic. Unfortunately both the vernacular and technical terms carry erroneous connotations and moreover imply a dichotomy that does not exist. It is properly assumed that the body temperature of the poikilotherm varies directly with that of the environment. Even though ecologists have long recognized the fact (see Chapman and coauthors, 1926, for example), it is not so generally understood by others that the environment includes not only the air and the substratum, but solar radiation as well, and that animals avail themselves of the great variations in temperature to be found in time and space to avoid extremes and to exercise a measure of control over the thermal level of the body.

When confined in the laboratory cage a reptile cannot control its temperature, which may indeed approximate "that of the surrounding atmosphere" as stated in many texts. When active under natural conditions it often maintains the body at a thermal level that is higher than that of man and many other mammals. With their elaborate mechanisms to produce heat internally, the birds and mammals are appropriately characterized as endothermic, unlike the reptiles that derive their body heat from external sources, and may aptly be termed ectothermic, as Cowles (1940) suggests. But there are various degrees of perfection in the state of endothermy, and the active, ectothermic lizard in the desert is scarcely to be called "cold-blooded." Mazek-Fialla (1941) points out that internal factors play an important role in the maintenance of body temperatures by poikilothermic animals.

The body temperature of the terrestrial diurnal reptile may be lowered by the loss of heat through radiation, convection, or by the evaporation of body fluids; it may be either raised or lowered by the conduction of heat to or from the substratum or the air, or its temperature may be raised by the absorption of radiant heat from the sun.

While there may be a very rough direct correlation between changes in the body temperature of the lizard and those of the air or substratum, it is only under special conditions in the laboratory (or in such unusual conditions as prevail in caverns or aquatic situations) that the reptile's temperature is identical with that of either component of its environment. Within a few seconds an active lizard in its native habitat may traverse an area where portions of the ground surface are shaded, partially shaded, or exposed to full sunlight. In desert regions the tem-

perature of the substratum at various points along the lizard's path may vary as much as 20 to 30° C. Variations in air temperatures are unlikely to be of this magnitude, but as part of the lizard's environment, they may shift from moment to moment, particularly if a lizard is moving about in and out of rock crevices.

At high altitudes where the air is less dense, direct solar radiation may raise a reptile's temperature to levels many degrees higher than that of the air. Differences as great as 29° C. between air temperatures and the body temperatures of reptiles living at altitudes of 4000 meters in the Caucasus have been reported by Strel'nikov (1944). This statement may be open to some doubt, although there is no question concerning the ability of a basking lizard to raise its temperature far beyond that of the surrounding air.

Thus it is not practical to correlate a reptile's body temperature with the composite of temperatures that exist in various elements of its natural environment. It has been recognized (Cowles and Bogert, 1944), however, that reptiles, not unlike the dune insects described by Chapman and co-authors (*supra cit.*), take advantage of the different thermal levels in their environment. They bask or select suitably warm areas of the substratum to raise the body temperature, or retreat to shade or burrows to lower it. Additional control can be exercised by orienting the body with respect to the sun's rays when basking, and most reptiles resort to respiratory cooling at levels near the critical maximum. Thermoregulation by means of behavior is characteristic of all reptiles thus far studied. It should be emphasized, however, that basking plays a dominant role in the existence of diurnal lizards, whereas nocturnal reptiles rely largely upon the heat of the substratum as a source of body warmth.

For these reasons it is difficult to simulate environmental conditions in the laboratory. After experimenting with various types of apparatus Cowles and Bogert adopted the expedient of setting up open cages in the field where animals could be confined as well as observed in a close approximation of the native environment. Under these conditions it was possible to ascertain the methods utilized by reptiles in adjusting the body temperature. Criteria of use in recording significant thermal levels of the body were defined, and extreme tolerances, as well as the normal activity range and its mean or ecological optimum, were obtained for several species of snakes and lizards inhabiting the Southwest. It became apparent, however, that a more extensive comparative study would be required before a satisfactory understanding of the thermal requirements of reptiles could be attained. Accordingly, as a necessary step in the plan of investigation, field work has been undertaken in various parts of North America with the object of accumulating a large amount of accurate data concerned with the body temperature of reptiles in a variety of habitats. The results of part of the work thus far carried out are summarized in this report, and some more or less tentative conclusions concerning the implications of the data are presented.

METHODS

The results reported by Cowles and Bogert in 1944 were obtained, as noted above, by recording body temperatures of lizards confined in various types of cages set up in the field. Possible limitations in this technique were recognized, however, and various means were considered to check the results. One of the simplest methods consists in shooting lizards in the field, and recording body temperatures immediately. Some species of diurnal lizards are relatively abundant, and data for representative series thus recorded should provide a reliable index to the variation in the body temperatures of any given population. Consequently this method was adopted.

Collecting was done with a single-shot target pistol with a ten-inch rifled barrel, using "long rifle" .22 dust shot for am-

munition. Whenever possible lizards were shot at a range of 2 to 4 meters. The shot at this distance was sufficiently scattered that usually only a few penetrated, the number, of course, depending upon the distance and the size and orientation of the lizard. Specimens shot at distances closer than 2 meters often had to be rejected owing to the copious flow of blood and the cooling that resulted from evaporation.

Because sensitive thermocouple equipment was too bulky to be carried conveniently in the field, suitable thermometers had to be designed and these were made by the Schultheis Corporation in Brooklyn. The instrument obtained was a small mercury thermometer 18 cm. long, with a bulb 7 to 12 mm. long and only 2 mm. in diameter that could easily be thrust into the cloaca of even the smallest lizard. It was calibrated in divisions of .2° C. between the range of 0 and 50° C. Repeated experiments indicated that it was sufficiently sensitive to reach equilibrium within approximately 15 seconds. Thus it was possible to record the temperature of a lizard within thirty seconds or less after it was shot.

Snakes as well as nocturnal and secretive lizards can usually be collected by hand without shooting them. However, few of them can be taken in sufficient numbers at a single locality to provide a sample large enough to indicate reliably either the range or the mean, unless several seasons are devoted to the work. The data thus far obtained in sufficient quantity are mainly for the common species, especially the scaly lizards (*Sceloporus*), and the whiptails or race-runners (*Cnemidophorus*), although species of *Holbrookia, Uta,* and *Uma* have been secured in fair quantities.

Representative series have been obtained from populations in Florida, Arizona, California, New Mexico, Honduras, and various states in Mexico, including the subtropical lowlands of San Luis Potosí. The present paper, however, will be limited largely to a consideration of data secured at the Archbold Biological Station in Highlands County, Florida, and at the Boyce Thompson Southwestern Arboretum in Pinal County, Arizona. The records from Florida were all secured from lizards shot in the "rosemary scrub" (Carr, 1940), whereas those in Arizona were all from lizards taken in the area mapped by Nichol (1937) as the "desert grass (mesquite)" where the saguaro cactus and mesquite are the conspicuous plants.

The gross climates of the two localities are markedly different, although precise data for neither are available. However, summaries of records from nearby places have been recorded (Kincer, 1941), and these provide a rough index to the differences:

	Pinal Co., Arizona (Florence, 22 yr. record)	Highlands Co., Florida (Avon Park, 37 yr. record)
Average Temp., January	10.3° C.	17.6° C.
Average Temp., July	32.2° C.	27.5° C.
Maximum Temp.	47.2° C.	38.9° C.
Minimum Temp.	−11.7° C.	−6.1° C.
Growing season, days	250	347
Rainfall	262 mm.	1298 mm.

In other words, the mean temperature for the Arizona locality is approximately 4.7° C. higher during the summer months, and 7.3° C. lower during the winter, while the rainfall is five times as great in Florida where the growing season is slightly less than a hundred days longer.

At both the Archbold Station and the Boyce Thompson Southwestern Arboretum the lizards commonly seen were those of the genera *Cnemidophorus* and *Sceloporus*. In Florida, where data were secured in May and June and later in August and September, *C. sexlineatus* and *S. woodi* occurred side by side, often in clearings or along roads and fire lanes. Both species are largely terrestrial although *S. woodi* occasionally climbs posts or trees.

At the Arboretum in Arizona lizards were shot in August and September during the unusually dry and hot summer of

1945. The common species here were *C. tessellatus* and *S. magister*. The former, as in the case of its congener in Florida, was almost entirely terrestrial, and most abundant in the dry, sandy washes. The *Sceloporus* on the other hand commonly inhabited rock walls in shaded or partially shaded locations. It was rarely seen basking, and seemed to be actively foraging only during the early morning and late evening.

Other data recorded in addition to the cloacal temperatures of the animals taken in Arizona included the time of day, the temperature of the substratum where the lizard was first seen, and the temperature of the air 5 cm. above this point. Also the sex was noted, and shortly after death each lizard taken at the Arboretum was weighed. Only the time of day, the sex, and the body temperature were recorded in Florida.

It is of interest to point out that the Arizonan species in both instances is larger than its congener in Florida. *Sceloporus m. magister* reaches a maximum snout-to-vent length of approximately 140 mm. whereas *S. woodi* rarely reaches 55 mm. *Cnemidophorus tessellatus*, with a maximum snout-vent length of 95 mm., is roughly 20 mm. longer than *C. sexlineatus*.

Results and Their Implications

The data secured at the two localities are corroborated by additional data not presented here, but now available for closely related species inhabiting other portions of North America. The results of the work at the Archbold Biological Station and the Boyce Thompson Southwestern Arboretum are most readily summarized in table I. Inferences that may be drawn from these data follow:

Table I. *Summary of data for body temperatures of lizards and of air and substratum temperatures, in °C., recorded in Florida and Arizona*
(Extremes are given in parentheses below the mean and its standard error)

	Sceloporus		*Cnemidophorus*	
	S. magister (Arizona)	*S. woodi* (Florida)	*C. tessellatus* (Arizona)	*C. sexlineatus* (Florida)
Number	10	42	33	12
Mean, body temps.	34.9±.56 (32.0–37.0)	36.2±.25 (32.0–39.2)	41.3±.24 (37.4–43.5)	41.0±.47 (38.5–43.0)
Coefficient of variation	5.09	4.53	3.30	3.93
Mean, body temps. ♂♂	33.5±.88[a] (32.0–34.8)	36.3±.42 (32.5–38.8)	40.8±.45 (37.4–42.6)	40.9±.36 (39.5–42.0)
Mean, body temps. ♀♀	36.1±.43 (34.0–37.0)	36.0±.35 (32.0–39.2)	41.5±.79 (39.3–43.5)	41.1±.66 (38.5–43.0)
Mean, body temps., May–June	—	35.9±.40 (32.0–39.2)	—	40.5±.70 (38.5–42.5)
Mean, body temps., Aug.–Sept.	—	36.5±.32 (32.5–38.8)	—	41.3±.33 (40.5–43.0)
Mean, air temp. records[b]	32.5±.78 (29.3–38.5)	—	33.6±.43 (29.2–39.2)	—
Coefficient of variation	7.61	—	7.32	—
Mean, substratum temp. records	32.61±.13 (29.6–40.5)	—	41.3±1.07 (32.5–58.9)	—
Coefficient of variation	10.36	—	14.86	—

[a] Only 3 males from Pinal County; inclusion of additional data from Yavapai County, Arizona, results in means of 35.2° C. for males and 35.0° C. for females.

[b] Substratum temperatures were recorded as nearly as possible at the spot where lizards shot were first seen. Air temperatures were recorded 5 cm. above the spot, or to one side when lizards were on walls or trees.

(1) Differences between the sexes as far as body temperature preferences are concerned appear to be nil. When samples are adequate there is no significant difference between the mean body levels of males and females. Consequently the temperature samples can be analyzed with reference to other factors without considering the sexes separately.

(2) Seasonal differences in body temperatures are not indicated for the series taken in Florida, even though air temperatures, as recorded by the Weather Bureau, are lower in spring than they are in late summer. Using the formula for the comparison of small samples (Simpson and Roe, 1939, p. 212) the difference of 0.8° C. between mean body temperatures of the series of *C. sexlineatus* taken in the spring and those taken in late summer, and the difference of 0.6° C. between the means for the two series of *S. woodi* are shown not to be statistically significant. In each instance P is greater than .2.

The lack of any seasonal correlation is conveniently shown by means of a scatter diagram (fig. 1) wherein the time of day has also been indicated. The series of 42 records for *S. woodi* when plotted shows no indication of any diurnal rhythm in body temperatures, although few lizards were secured in the afternoon. The paucity of afternoon records results in part from the fact that lizards are more active during the morning hours, but also reflects the activities of the collectors, who were not infrequently working on another project in the afternoon.

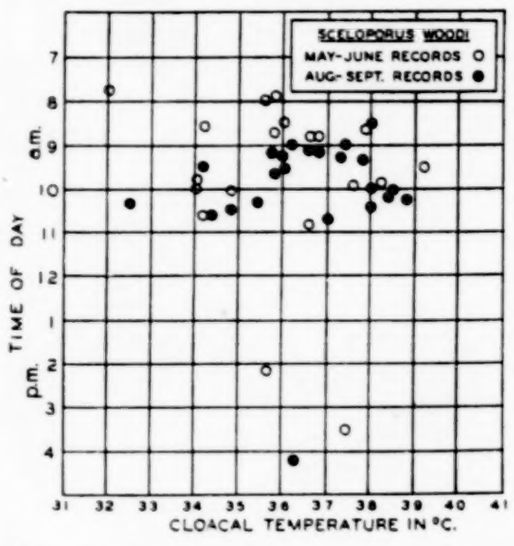

FIG. 1. Scatter diagram showing the absence of correlation between seasonal or diurnal changes in air and substratum temperatures and the body temperatures of 42 lizards (*Sceloporus woodi*) taken at the Archbold Biological Station, Highlands County, Florida.

Despite the inadequacy of proof at present, it seems manifest that diurnal lizards bask in the morning until the body temperature is raised to the threshold of the normal activity range. Thereupon, they become able to carry out their routine activities. Prolonged activity in direct sunlight may result in their reaching the upper limit of their normal activity range. This may be 5 or 6° C. below the critical maximum or potential lethal (Cowles and Bogert, 1944, p. 287), although the data assembled thus far indicate that lizards rarely remain in direct sunlight or on a hot substratum until their limit of voluntary tolerance has been reached. Ordinarily they evidently seek the shade or some cooler spot where heat can be dissipated and the body temperature lowered to a point that probably approximates the mean of the normal activity range.

If weather conditions are near the optimum a lizard is thus able to remain abroad for the better part of the day. On the other hand, retirement to cooler depths underground may be necessary if ground and air temperatures are too high. With the sun at the zenith on a clear summer day few reptiles are abroad, especially in desert regions. Those seen usually are foraging in shaded areas. Similarly, cold weather or the lack of adequate sunshine may result in a lizard's being unable to raise its body temperature to a level that will permit normal activity. If the air and substratum are below the lower limit for the basking range, not yet ascertained with precision for any reptile, the lizard may not emerge. But on occasional days in spring or fall

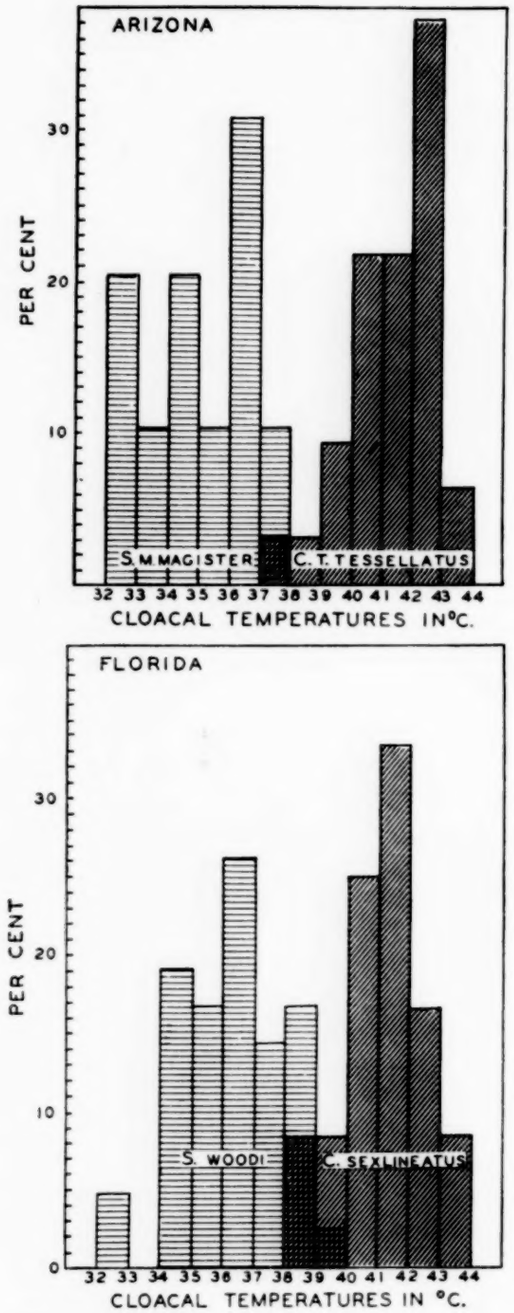

FIG. 2. Histograms showing the distributions of body temperatures under field conditions of four species of lizards belonging to two genera, *Sceloporus* and *Cnemidophorus* (data from table 1), illustrating similarities in body temperature preferences of lizards of the same genus in widely different climatic regions and dissimilar habitats, as well as differences between body temperature preferences of lizards in different genera, but living in almost identical habitats. Cloacal temperatures plotted between limits indicated in °C. on the abscissa, and percentages of the total sample for each species are shown on the ordinate.

lizards appear to bask without ever raising the body temperature to the threshold level of the normal activity range, even though the lizard may be capable of locomotion and able to seek shelter upon the approach of an enemy.

On cloudy or overcast days, even in summer, lizards with high thermal preferences may find it difficult to raise the body temperature to the required level. A small series of whiptail lizards taken in Arizona during the course of two cloudy days had a mean temperature almost a degree lower than the mean for the entire series. The difference is perhaps little more than suggestive, but it may be noted in figure 2 that the curve for the desert whiptail (*C. tessellatus*) tends to be skewed toward the upper end of the scale, whereas the curve for the Floridian species of the same genus is skewed toward the lower end. This may reflect the absence of clouds at the Arboretum, and the frequent occurrence of thunderstorms and overcast days at the locality where the Floridian series was taken.

(3) Size, or body volume, as a factor in the maintenance of temperatures within the normal activity range must be considered from the standpoint of the species as well as the ontogeny of the individual. By reference to figure 3, it may be observed that there is no evidence that juveniles of *Cnemidophorus t. tessellatus* have mean temperatures that differ from those of adults. But there is some indication that juveniles are somewhat more stenothermic than adults as far as body temperatures are concerned. The range for 12 juveniles weighing less than 7 grams falls between the limits of 39.3° and 42.3° C. (mean 41.2° C., coefficient of variation 2.31) in contrast to the range for 21 specimens weighing 9 grams or over, which falls between 37.4° and 43.5° C. (mean 41.3° C., coefficient of variation 3.73). These differences may possibly reflect greater sensitivity to heat on the part of juveniles, or the ability of a smaller animal to raise or lower its temperature with greater rapidity.

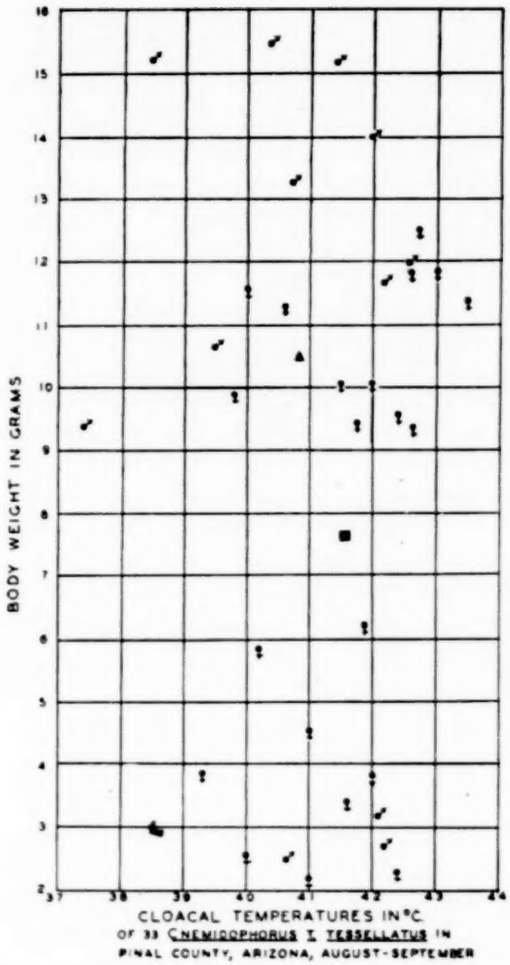
BODY WEIGHT IN GRAMS
CLOACAL TEMPERATURES IN °C.
OF 33 CNEMIDOPHORUS T. TESSELLATUS IN
PINAL COUNTY, ARIZONA, AUGUST-SEPTEMBER

FIG. 3. Scatter diagram, showing the absence of any correlation between size or sex and preferred body temperatures in 33 whiptail lizards (*Cnemidophorus tessellatus*) taken in the field. The mean temperature and body weight for males are indicated by the triangle, and for females by a square. Juveniles are less variable than adults, suggesting that they are more sensitive to changes in the thermal level of the body, as reflected by cloacal temperatures.

The distributional and habitat data for lizards of the genera *Sceloporus* and *Cnemidophorus* suggest that a rough correlation exists between climate and adult body size. There are exceptions as well as overlaps in distribution, but in general the larger species inhabit warm regions at low elevations, whereas species from cooler regions or higher elevations tend to be small. In the San Jacinto Mountains of Riverside County, California, four species of scaly lizards (*Sceloporus*) occur. The largest, *S. magister,* with a maximum body length of 140 mm., is absent from the coastal region, but ranges from the desert foothills eastward into the warm, arid, Coachella Valley with its sparse vegetation. Above the desert foothills, *S. occidentalis* with a body length of 90 mm. occurs between approximate elevations of 4000 to 6000 feet, although on the cooler coastal side of the mountain it is a common lizard at much lower elevations, down to the sea. The smallest of the four, *S. graciosus,* with a maximum body length near 65 mm., is restricted to elevations principally above 5000 feet, although it may descend somewhat lower in cooler canyons on the western side of the mountain.

Factors other than size appear to be involved in other distributions, however, since the fourth species, *S. orcutti,* occurs not only on the coastal side, but has an altitudinal distribution ranging from canyons on the very edge of the desert at 500 feet in the foothills to elevations exceeding 7000 feet. It attains a body length of 109 mm., intermediate between *occidentalis* and *magister.* The nature of the pigmentation and the type of scalation are probably additional factors of importance in this distribution. The largest species is lightest in coloration and has relatively large mucronate (with a *mucrone* or projecting spine at the posterior end, as a continuation of a median keel) scales, whereas the skin of *graciosus* at the higher elevations tends to be dark slaty black, and the scales are small, with less pronounced mucronations. *S. occidentalis* in the intermediate zone is roughly as dark as *graciosus* but has somewhat larger scales. The fourth and least restricted species, *orcutti,* has a relatively dark pigmentation, with the size of the scales roughly intermediate between those of *magister* and *occidentalis.* All four species are susceptible to pigmentary changes, being darker at low temperatures.

(4) Differences in the micro-habitat selected by two lizards with different body temperatures are reflected in the air and

substratum temperatures (table I) recorded in Arizona. A difference of 1° C. between the means for air temperatures of the two may not be statistically significant although it could result in part from the fact that at the time field work was carried on at the Arboretum *Sceloporus magister* occurred only in shaded habitats, and was not often abroad when air temperatures were high, but still tolerated by *Cnemidophorus*. More probable, however, is the effect of the warmer substratum preferred by *Cnemidophorus* on the air 5 cm. above it. The thermometer, as well as the lizard, would be warmed by heat radiating from the ground, as well as transferred to the lower layers of air by direct conduction. On windy days the difference between air temperatures at various elevations in the shade and in the sun may be reduced to zero, but on other days air temperatures taken a meter or so above the ground are lower than those taken at a distance of 5 cm. Consequently the mercury column of a thermometer held 5 cm. above ground exposed to the sun would be raised not only by heat conducted from the adjacent substratum, but by heat radiating from the ground. Wellington (1949) points out the desirability of exposing the instrument to conditions equivalent to those of natural bodies.

It was manifest that *Cnemidophorus* was spending more time than *Sceloporus* in areas exposed to direct sunlight. Undoubtedly the temperature of the substratum affects the thermal level of the lizard, but the similarity of the means calculated for body and substratum temperatures of *Cnemidophorus* can scarcely be construed as evidence that the bulk of the lizard's body heat is derived from the substratum. Part of the time that it is active the lizard must be receiving heat directly from solar radiation as well as from the substratum. Moreover, the body temperatures of *Cnemidophorus* are not closely correlated with those of the substratum where they were killed. The desert whiptail lizard is an extraordinarily active creature, almost constantly in motion, passing in and out of shaded spots where it forages. Data secured at the Arboretum indicate that it may be taken on terrain with a surface temperature as much as 14° C. lower or 16° C. higher than its body temperature.

(5) The similarities between the means and extremes of body temperatures recorded for lizards of the same genera from regions with gross climates as different as those of Arizona and Florida are perhaps the most striking facts that emerge from the data presented. Differences between means for species in the same genus are not statistically significant ($P > .1$ in both cases). Additional data from populations of other species of the same two genera in Mexico confirm the inference that lizards of the same genus, whether they live in warm or cool regions, tend to have similar body temperature preferences. However, there are species of *Sceloporus* in Mexico with somewhat higher thermal preferences than those described here, and in some instances the differences between means for these species are statistically significant. Nevertheless, closely related forms, even though they are sometimes placed in separate genera, tend to have thermal preferences, or normal activity ranges, that are extraordinarily close, despite marked dissimilarities in their habitats.

Discussion

These investigations provide further proof of behavioral thermoregulation in reptiles. In fact, under natural conditions rather than in cages set up in the natural environment, lizards are able to exert an astonishing amount of control over the thermal level of the body; in their active states they are far less poikilothermic than is usually assumed. Too much emphasis has been placed on the results of experimental work in the laboratory where ways of controlling the body temperature were available to the experimenter but not to the animals used. The literature is filled with statements

concerning the "temperature" of reptiles, or of "the environment," with nothing either implicit or explicit concerning precisely what is meant. Too much attention has been devoted to "lethal temperatures" despite the lack of any satisfactory criteria for determining the death point, and the relative unimportance ecologically of lethal body temperatures among animals so eminently capable of thermoregulation.

Experiments designed to test the effects of individual factors (air and substratum temperatures and relative humidity) are not without value, although some interpretations of such data have resulted in conclusions of dubious validity. Herter (1940), for example, designed an apparatus (described in 1934) with a thermal gradient in the substratum. Reptiles placed in this *"Temperaturorgel"* were permitted to orient themselves, and to select the levels preferred. Temperatures of the substratum below the middle of the reptile were recorded at unstated intervals, and the resulting data were analyzed statistically, with the mean given as the "Vorzugstemperatur" or "V.T."

Inasmuch as the effects of direct solar radiation in the native habitats of the animals used were ignored, and since such factors as skin thickness, body size and shape affect the thermal level of animals heated from below, Herter's findings are one-sided in relation to the preferred (or eccritic, from the Greek, "pick out," as suggested by Gunn and Cosway, 1938, for those who object to the anthropomorphic connotations of "preferred") body temperatures of reptiles. It must be emphasized at this point that the preferred substratum temperature (or V.T.) of Herter is by no means the same as the preferred body temperature, henceforth referred to as the PBT, to indicate the "normal activity range," a zone of preference, the mean of which is readily expressed quantitatively. The value of this distinction is perhaps best illustrated by pointing out that the PBT of a small juvenile lizard of a given species is the same as that of a large adult, while the V.T. (which will be rendered below as the "preferred substratum temperature," abbreviated as PST) is significantly lower than that of the adult. The reason for this is that a warm substratum heats a small individual faster than a large one, the difference depending in part on the temperature and rate of movement in the air.

The PBT and its mean or optimum are fixed by heredity. However, Herter places the PST at the level of the local population for probable reasons noted below, whereas data for North American lizards indicate that uniformity in the PBT is at the species level, and moreover that closely related species have similar, but not necessarily identical body temperature preferences.

Cowles and Bogert (1944, pp. 275–277) show that the mean body temperature of a lizard of moderate size may be 4° or 5° C. lower than that of the substratum at its point of contact with the lizard's body, when the substratum (a slab of slate was used in experiments) is the source of body heat. The temperature of the air surrounding the reptile exerts relatively minor effects, although the amount of heat transferred from the substratum is unquestionably influenced by the conductivity index of the sand, rock, or metal upon which the lizard is placed.

Consideration of these factors explains the relatively high means obtained for the PST of Old World reptiles as compared to those for the PBT in North America. Herter reports the PST of two North American snakes, *Agkistrodon contortrix* and *A. piscivorous,* to be 34.42° and 35.34° C., respectively. Both species are restricted to relatively cool habitats in eastern United States. Cowles and Bogert, whose studies were largely restricted to reptiles inhabiting Coachella Valley, one of the warmest, dryest, sections in America, report 33.0° C. as the mean for the PBT of the red racer (*Masticophis*

flagellum), the most heat tolerant of the serpents tested.

More definite evidence is available for *Agama stellio,* a lizard from the Egyptian Desert reported by Herter to have a PST of 45.59 ± .33° C. This is at least 3° C. higher than the maximum body temperature voluntarily tolerated by lizards in the Southwestern deserts, and well above the mean for the normal activity range of any reptile thus far tested. However, Scortecci (1940, p. 87), who recorded body temperatures of *Agama stellio* in the Libyan Desert at approximately the same latitude as Egypt, reports the mean to be 33.4°, or slightly more than 12° C. lower than the PST reported by Herter. Thus it may be seen that figures obtained by recording the temperature of the substratum at the middle of the animal in the gradient chamber may not even approximate the actual body temperatures. Moreover a higher substratum temperature would be required to raise a large reptile to its preferred body temperature than would be required to raise a small one. Hence, assuming for a moment that no other factors are involved, larger reptiles with similar mean body temperature preferences would tend to select higher levels in the thermal chamber. The size factor alone could readily account for the lower temperatures preferred by juveniles in the chamber, and it is probable that the differences between local populations that Herter reports can be attributed to differences in mean adult size, a character that is often subjected to selection. Thus, data obtained by Herter's methods reflect the effects of selection on morphological characters rather than adaptations in the neurophysiological mechanism involved in body temperature control.

Findings for North American lizards thus far studied suggest, however, that there are minor adaptive changes in the PBT. In southern California Cowles and Bogert (1944, p. 282) detected slight differences in the mean body temperature preferences of three species of horned lizard (*Phrynosoma*) living respectively in (a) the mountain, foothill, coastal regions, principally in the areas covered by a relatively dense chaparral, (b) the rocky or sandy deserts with sparse vegetation, and (c) the dune areas of the desert. The mean body temperatures of the latter two species are almost identical, but that of the species in the cooler coastal region is 2° C. lower. In a previous section of this paper, it is noted that means for body temperature of two species of *Sceloporus* from widely different habitats are nearly identical, and the same holds true for two species of *Cnemidophorus*. But minor differences have been noted between species of these and other genera.

Quantitative data concerning the habits in nature or under suitable conditions in the laboratory are not offered by Herter, and they are not yet available for North American reptiles. However, it is manifest from the data secured in Florida that two lizards in the same habitat can, by means of their behavior or habits, maintain body temperatures at mean levels that are significantly different. Thus body temperatures are the result of an interaction of the effects of (a) habits and (b) habitat, and it is only in a very loose sense that any correlation can be said to exist between the PBT and the habitat.

It has been shown (Bogert, 1939) that there is a rough correlation between vertical and latitudinal distributions of several wide-ranging species of reptiles inhabiting western United States. However, species with limited ranges cannot be included in the picture. It seems obvious that the distributions of those with specialized habits are dependent, not only upon thermal factors, but upon others of more basic specific importance. The granite night lizard (*Xantusia henshawi*), for example, is restricted to regions of exfoliating granitic rocks in California and Baja California, and occurs only where such flaking provides the sort of shelter for which its flattened body seems peculiarly well adapted.

Body temperature regulation and habitat selection

Aside from specializations mentioned above, the inheritance of either high or low preferences in the range and mean of the thermal level of the body imposes restrictions in the selection of habitats. In a hot, dry, desert region with sparse vegetation, a diurnal terrestrial reptile with an innate predilection for relatively low body temperatures would find it difficult to remain abroad for sufficiently long periods of time to fullfil its needs for sustenance, reproduction, and the avoidance of predators. Conversely, a reptile with a body temperature preference much exceeding 38° C. can find conditions suitable to maintain such a high level only in regions of relatively sparse vegetation, where direct solar radiation and high substratum temperatures are the rule for large portions of the year. Humid regions, with prevailingly overcast or cloudy days, and the dense forests that so often accompany these climatic conditions, are unsuitable habitats for reptiles with a high body temperature requirement.

The reptile's ability under natural conditions to control its body temperature by means of behavior, therefore, implies the necessity for the selection of habitats wherein the preferred thermal level of the body of the species can readily be maintained during most of the season of normal activity. Under field conditions direct solar radiation, the effects of which were ignored in Herter's experiments, is of considerable importance. Most diurnal lizards, especially those with high body temperature preferences, notably teiids (only *Cnemidophorus* in the United States) and iguanids, depend to a large extent upon basking as a means of raising the body to the levels dictated by hereditary factors. The abundance in the American Southwest of lizards that prefer relatively high body temperature levels may result in part from such historical factors as ecological barriers or routes from a relatively recent center of dispersal. But as a group iguanids are heliotherms, and specializations along this line probably occurred early in the evolution of the family. Their inability to penetrate regions now unoccupied in North America is reflected not so much in the mean temperatures of these regions as in the availability of direct solar heat. Such secretive lizards as skinks (principally *Eumeces* in North America) with low body temperature preferences approximating 30° C. are dominant in Florida and the Gulf Coast, in contrast to the Teiidae and Iguanidae (several genera in the United States), which are far more abundant in the arid regions of the Southwest.

California with 34, Arizona with 35, New Mexico with 26, and Texas with 40 are the only states inhabited by more than two dozen species of lizards. Significantly these are the states in or on the edge of a region in which the average annual number of clear days over extensive areas exceeds 180 (Kincer, p. 742, map). Admittedly several other factors including the diversity of the terrain are involved. The presence of so many species can be attributed in part to the variety of habitats in the desert, mountain and coastal regions of most of these states. The mean annual temperature doubtless is of importance, but considered alone it does not account for the abundance of lizards (especially iguanids) in the Southwest. Florida, with an average annual temperature higher than that of most portions of Arizona, has but 13 native species (exotics are not included) of lizards, in contrast to 35 recorded for Arizona. Most of the other Gulf Coast states east of Texas, where the average annual number of clear days falls below 140, but with mean annual temperatures 15° to 20° F. higher than those of Nevada, Utah and Colorado, are inhabited by approximately half as many species of lizards as the latter states.

Vertical and latitudinal distributions of reptiles, therefore, are the result of so many factors that any high degree of correlation between these and preferred body temperatures would not be expected.

Within limitations resulting from hereditary preferences, adaptive modifications in such characters as size, shape, pigmentation, and habits, permit closely related lizards to maintain similar body temperature levels and still occupy different habitats.

The evolution of endothermy

One of the problems confronting adherents of natural selection lies in the difficulties encountered in explaining the evolution of complex mechanisms, many parts of which would seemingly have to be evolved separately and yet be of little value to the animal until each is integrated and functioning with the mechanism as a whole. Offhand it might appear difficult to account for the acquisition and perfection of the mechanism for internal heating by endothermic vertebrates. Presumably this had its antecedents in their ectothermic reptilian ancestors, and the ability of some existing reptiles to maintain high, relatively constant, body temperatures when active, provides information of value in accounting for the evolution of the separate elements involved in the mechanisms of thermoregulation in the birds and mammals.

Behavioral control of body temperature in the majority of reptiles implies a rather high degree of sensitivity to changes in the internal environment. The normal activity range or PBT, the mean of which may be termed the optimum, permits minor fluctuations inherently necessary in an animal dependent upon extrinsic heat. Departures from this range, however, elicit locomotor responses, and compensatory adjustments result from the selection of colder or warmer positions in the environment (which must be defined to include direct solar radiation), depending upon whether the body temperature has risen or fallen below the normal range. It has been demonstrated that a central coordinating mechanism or "thermotactic center" exists in some endotherms, and Martin (1930) has emphasized the proofs that the central mechanism of thermoregulation is itself sensitive to temperature. The precise location of the center is not established with certainty although it seems definitely to be located anterior to the region of the interbrain in mammals, and there are reasons for believing it to be located in the *tuber cinereum*.

Little effort has been devoted to experimental studies of reptiles probably because it was erroneously assumed that they are not particularly sensitive to temperature changes. Recently, however, Rodbard (1948) noted that the blood pressure in the turtle varied with the temperature of the animal. By inserting a silver wire connected with a water reservoir into the brain of the turtle, he found that warming the brain caused an immediate rise in arterial pressure, while cooling caused it to fall. However, slight thermal variances caused covariance in the blood pressure only when the wire was in or near the hypothalamus (which includes the *tuber cinereum*), and not when it was in the medulla or in the olfactory or optic lobes. Direct evidence for a temperature-sensitive center in the hypothalamus of a cold-blooded animal is of particular interest from an evolutionary standpoint since it indicates that the central mechanism of thermoregulation in ectotherms is located in the same general region of the brain that it is in endotherms. Inasmuch as the latter have evolved from the former, this might have been anticipated, although the central nervous mechanism for thermoregulation in endotherms is far more complex, requiring coordinated but antagonistic thermogenic and thermolytic subcenters, each sensitive to appropriate, direct, and reflex stimulation.

Rodbard points out that the discovery of thermal sensitivity in the hypothalamic region of an ectotherm makes it possible to correlate the information now available for a large number of seemingly unrelated functions attributed to this miniscule portion of the brain. "It has been considered the head ganglion of the auto-

nomic nervous system, responsible for the regulation of body temperature, blood pressure, respiration, appetite, the diurnal rhythm of sleep and wakefulness, the sexual cycle, and the control of the metabolism of sugar, fat and water." Thus, as Rodbard perceives, these functions may be considered as parts of an integrative mechanism, and the calorigenic properties of adrenalin and thyroxin suggest that an intimate connection with the endocrine system may be involved as well. He speculates that Triassic reptiles, "having developed the ability to withstand large temperature changes," gave rise to the early mammals, and that later, in the Jurassic, "another group of reptiles, which may have increased their diurnal temperature range still more, gave rise to the ancestors of the modern birds."

Even though Rodbard mentions that optimal body temperatures are regulated by locomotor responses, he appears to have overlooked the significant fact that reptiles manage to maintain the body temperature within a relatively narrow "normal activity range," with fluctuations of only three or four degrees above or below the mean. This would appear to be of especial importance if any tenable theory can be advanced concerning the evolution of an integrative mechanism of thermoregulation. It would seem vitally necessary that various elements involved in the complex mechanism be at least partially integrated in advance of the acquisition of a truly endothermic metabolism. Behavioral control of body temperature by some of the more specialized reptiles would have permitted selection of many of the essential features involved in the integration before either mammals or birds came into existence.

It need not be postulated, however, that the primitive mammals were necessarily endothermic. In fact, experimental evidence concerning such existing mammals as the monotremes (*Tachyglossus*) and the sloths (*Choloepus* and *Bradypus*) indicates that these mammals are virtually ectothermic (Martin, *supra cit.* and Britton and Atkinson, 1938). It may safely be assumed that the present perfection of thermal adjustment in the higher mammals and birds was acquired gradually, and there is no reason to doubt that the endothermic condition in each evolved independently.

Thermoregulation and dispersal

Endothermism implies a measure of emancipation from the environment, and the restriction of such animals as the sloth and spiny ant-eater to tropical regions is readily explained by their failure to tolerate fluctuations in the temperature of the environment. According to Martin (*supra cit.*) *Tachyglossus* has no sweat glands; it does not pant when it is hot, although it shivers violently with cold. Its body temperature varies 10° C. as the external temperature rises or falls from 30 to 5° C., and the animal succumbs with brief exposures to an external temperature of 35° C. Obviously such an animal would fail to survive in the inaptly named "temperate zone," which in reality is a region of thermal extremes; the tropics, often thought of as being "warm regions," are more accurately defined as regions of relatively constant temperatures, only moderately high as compared with summer means in the desert regions of the temperate zone.

Darlington (1948) has advanced excellent arguments for the view that cold-blooded vertebrates have dispersed from the tropics into the north temperate zone, rather than the reverse, as suggested by Matthew. Darlington speculates that "great groups of animals rise to dominance in the largest and most favorable areas, which for cold-blooded animals are in the tropics of the Old World, and disperse into less favorable climates and smaller areas, their dispersal being facilitated by the ability of dominant groups to enter cold and probably other inhospitable areas."

If it be assumed, on the basis of such evidence as Martin provides for *Tachy-*

glossus, that the more primitive animals are unable to cope with extensive fluctuations in the environmental temperature, there are definite advantages for them in a tropical environment. It is not readily clear, however, why dominant groups should disperse into less favorable climates, unless it be further assumed that, even in the tropics, a selective advantage is placed on modifications that provide greater control over the body temperature. These modifications may be essentially physiological or behavioral. As far as reptiles are concerned, the data now available suggest that the thermal levels characteristic of individual genera were probably established at an early state in their evolution, and that dispersals into regions of temperature extremes have been possible largely as a result of modifications in the habits, behavior, or body size. Large reptiles are restricted to the tropics or to insular, peninsular, and aquatic (crocodilians) environments because of the expense in time that would be required to control the body temperature by behavioral methods in regions where the thermal level of the environment is subject to extensive change. Owing to the thermal capacity of water, aquatic animals are not subjected to the extreme fluctuations encountered by terrestrial animals in the temperate zone.

It is probable that some form of behavioral control of body temperature was utilized by Permian reptiles. By the Triassic various specialized trends may well have evolved. Less progressive stocks retained a preference for relatively low body temperatures, but others, availing themselves of radiant energy, sought higher temperatures. It may be assumed that thermoregulation was achieved independently by various evolutionary lines and that those with higher thermal preferences gave rise to birds and mammals. Whether heliothermic lizard stocks with sufficient perfection in behavioral control to maintain body temperatures at levels slightly exceeding 38° C. (approximating those of mammals) evolved at that time is problematical.

It seems reasonably certain, however, that during the Cretaceous, or earlier, lizards underwent an adaptive radiation, evolving terrestrial, secretive, and subterrestrial groups roughly corresponding to families currently recognized, even though there were later radial evolutions in individual families. Preferred body temperatures were necessarily modified to suit special habitat preferences (or *vice versa*) but seemingly there was a fair amount of stabilization at the generic level, which may not antedate the Miocene. Snakes, with thermal preferences approximating those of fossorial lizards, could, as supposed, have evolved from a burrowing lizard stock (or stocks, since the group is probably polyphyletic). The "living fossils," including the tuatara, crocodilians, and turtles, as far as known, tend to have low heat tolerances and requirements, and survive as unprogressive stocks, the majority of them in aquatic, tropical, or insular habitats.

The advantages of high thermal preferences are manifest, since the rate of muscular activity, the velocity of nervous impulses, and many other bodily functions are increased two or three times [1] by a rise of 10° C. Lizards with the highest preferred thermal levels tend to be more active than those with lower levels. The whiptail (*Cnemidophorus*), with a mean body temperature approximating that of a rodent of similar bulk, is quite as rapid in its movements, and probably remains active for as much of the year as the mammal with similar hibernation needs. To be sure no reptile has managed to penetrate regions where the subsoil is permanently frozen, but the expense of endothermism in man is approximately 40 times as great (for bare existence at 15° to 20° C.) in terms of fuel consump-

[1] This is a rough approximation, of course, and recent studies of muscle apyrase systems (Steinbach, 1949) have sought to explain the rapid mobilization of energy by such animals as fish at temperatures approaching 0° C.

tion (Martin, *supra cit.*) as it would be in a reptile with as much body surface.

Emphasis in this discussion has been placed on special aspects of evolutionary trends. For the sake of completeness it may well be added that the evolution of the integrative mechanism of thermoregulation in endotherms was dependent upon structural modifications in the lungs, heart, and other organs, with concomitant improvements in modes of reproduction. Cowles and his co-authors (1945, 1946) have called attention to the problems posed by the apparent lag in the toleration of high temperatures by the male germ cells in the evolution of endothermy. Noteworthy too were the changes in the skin. The earliest amphibian retained the scales as well as the low thermal preferences of its aquatic ancestor. Modern terrestrial amphibians, although they react poorly to gradients in temperature (Noble, 1931, p. 421), are sensitive to changes in humidity and may rely largely upon moisture lost through the skin and the resultant cooling from evaporation to retain their low body temperatures. But the transition from the amphibian to the reptile required the acquisition of a relatively impermeable skin. Kirk and Hogben (1940) point out that without such an integument maintenance of osmotic stability would have been impossible, along with the regulation of a high grade metabolic and nervous activity. Fur, feathers, or sub-integumental adipose tissue, characteristic of the advanced endotherms, conserve heat by insulating the body, but would manifestly be highly disadvantageous to the ectotherm where heat is derived from external sources. It is significant that fat storage is within the coelomic cavity of ectotherms.

In summary, limited behavioral control is reflected in the behavior of modern amphibians, with low body temperatures maintained through the evaporation of moisture. Reptiles rely largely upon behavioral control, whereas in such primitive mammals as the spiny ant-eater, thermoregulation is accomplished through habits and behavior, aided by a limited ability to vary internal heat production. The more advanced endotherms not only vary production, but exercise a measure of control over heat loss by a variety of means, including behavior, moisture loss, and respiratory cooling.

Conclusions

1. Lizards of two genera studied under field conditions in Florida and Arizona maintain the thermal level of the body within relatively restricted normal activity ranges, with fluctuations from the mean rarely exceeding 3° C. No significant differences were detected in the thermal preferences of the sexes, nor between juveniles and adults; in Florida similar mean body temperatures were maintained in spring as well as in the fall. These results confirm statements by Cowles and Bogert (1944) that thermoregulation is accomplished by means of behavior.

2. Lizards belonging to the same genus tend to have similar, but not necessarily identical, mean body temperature preferences, even though they live in different habitats or climatic regions. Body size appears to be one of the factors commonly affected by selection in the reptile's adaptations for a particular environment, although scalation, pigmentation, body proportions, and doubtless other characters may often be involved.

3. Lizards belonging to different genera may live side by side in the same habitat, but by behavioral thermoregulation maintain significantly different thermal levels in the body. Nevertheless, hereditary preference for a rather definite mean body temperature imposes limitations in the selection of habitats under extreme conditions.

4. Behavioral control of body temperature in reptiles implies a rather high degree of sensitivity. It is suggested that the evolution culminating in the complex integrative mechanism of thermoregulation of endotherms was dependent upon advance integration of many of the elements in progressive reptilian stocks that

independently achieved thermoregulation through behavior. Two of these stocks gave rise to the birds and mammals, respectively, while others may have become further specialized in behavioral thermoregulation, evolving into the modern heliothermic lizards with preferred body temperature levels as high as those in most endotherms.

5. Darlington's (1948) view that groups of ectothermic animals "rise to dominance in the largest and most favorable areas," especially the Asiatic tropics, and disperse into less favorable areas suggests that restrictions are imposed on stocks unable to adapt themselves to extensive fluctuations in the environmental temperature, but that behavioral or physiological improvements in thermoregulation permit some groups to achieve dominance, and later to extend their ranges into regions inaptly termed the "temperate zones" but characterized by extensive daily and seasonal changes in the composite of environmental temperatures. Modifications in habits, behavior or body size permit lizards to find suitable habitats in the temperate zone and still maintain the body temperature preferred by the ancestral group. On the other hand, since bulk impedes the rate of change in body temperature, the larger ectothermic vertebrates are largely restricted to tropical, insular (and peninsular), or aquatic habitats. The expense in time that would be required to raise or lower body temperatures to the preferred levels would be prohibitive in regions where the temperature of the environment is subject to extensive changes.

Acknowledgments

I am indebted to Mr. Fred Gibson, Director of the Boyce Thompson Southwestern Arboretum, and to Mr. Richard Archbold and members of his staff at the Archbold Biological Station for innumerable courtesies extended in the course of my work at the respective institutions. Dr. Raymond B. Cowles and Dr. Ernst Mayr both read the original draft of the manuscript, and I am indebted to them for extraordinarily helpful suggestions.

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SELECTION OF AN UNFAVOURABLE GENE-COMPLEX

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Received March 18, 1949

INTRODUCTION

It has been the general basis of the theory of natural selection that genes or gene-combinations improving the "fitness" of a species will tend to increase in frequency within the species, while those which are harmful will tend to decrease, as a result of selection. But Fisher (1941) has shown theoretically that a gene affecting the breeding system but not species fitness can be strongly selected; that is, intense selective activity need not be accompanied by an increase in the survival value of the species.

This paper describes a breeding system in which there is selection of a gene-complex which affects that system and which at the same time reduces species fitness, and evidence is presented which shows that such a system probably exists in two recently discovered groups of populations of *Primula vulgaris*. Selection of unfavourable genes must be clearly distinguished from the purely fortuitous increase in frequency of such genes which Wright (1940) has shown to be possible in small populations.

THE NORMAL BREEDING SYSTEM IN *P. vulgaris*

The primrose, *Primula vulgaris* Huds., is one of the best known examples of heterostyly. Normally it exists in two forms, "pin" and "thrum," which differ in morphological and physiological characters of their flowers. As Darwin (1877) first showed, this dimorphism is the basis of an outcrossing mechanism.

A flower of a pin plant (fig. 1, a) has a long style bearing the stigma at the mouth of the corolla tube, half way down which the anthers are attached. A thrum flower (fig. 1, b) has the anthers attached in the mouth of the corolla tube, while the style is short and the stigma half way down the tube. The pin stigma is covered with papillae about five times as long as those of a thrum, while pin pollen has a diameter about two-thirds of that of a thrum.

Darwin (1877) showed that while crosses between pins and thrums ("legitimate") were fully fertile, those between plants of the same type ("illegitimate") had a much lower fertility, especially in the case of thrums. Crosby (1948) found in an experiment on the closely related *P. veris* that the pollination thrum × thrum gave only about one-fifteenth of the number of seeds given by thrum × pin (seed parent is mentioned first). Pin × pin was less infertile, giving about one-third of the seeds per pollination of a pin × thrum cross. Data for *P. vulgaris* are scanty, but the illegitimate thrum pollination seems to be as infertile as in *P. veris;* the illegitimate pin pollination may be almost fully fertile although Darwin's results, unfortunately given as seed weight, suggest that this is not so.

Data of Lewis (1942) and Tseng (1938) on other species of *Primula,* and of the author (unpublished) on *P. vulgaris* and *P. veris,* show that the basis of the infertility of illegitimate pollinations is probably differential pollen-tube growth. Although thrum pollen may germinate on a thrum stigma, it rarely penetrates the stigmatic surface and few tubes reach the ovary. Pin pollen germinates well on a pin stigma, which it readily enters; at first it grows quite well, though not so fast as thrum pollen; there is some evidence that the rate may slow down in the style. It seems clear that if a pin stigma is pollinated simul-

taneously with pin and thrum pollen, the latter will reach the ovary first. If the assumption of the slowing down of illegitimate pin pollen is correct, then thrum pollen should overtake pin pollen in a pin style even if the latter reached the stigma some hours, possibly more than a day, previously. Results are not yet available from experiments designed to test this.

The agents of primrose pollination have long been a matter of doubt. Bell (1902) vigorously attacked Darwin's hypothesis (1877) of cross-pollination, asserting that self-pollination was the rule, and Harrison (1931) also believed that self-pollination was important. But Knuth (1909) was convinced of the adequacy of insect agency for cross-pollination.

In a preliminary investigation I examined from normal populations 16 flowers of *P. vulgaris* and 20 of *P. veris*, including both forms. Each form usually had sufficient pollen of the other for all the ovules to be legitimately fertilized. The presence, sometimes in large amounts, of pollen of the same form showed that illegitimate pollination (presumably usually self) can easily occur, and this was specially true of pins. But the almost complete failure of thrum pollen to grow on a thrum stigma, and the probable advantage of thrum pollen over pin pollen in a pin style, mean that self-pollination is largely irrelevant, since so long as cross-pollination occurs as well, cross-fertilization will usually result. But where cross-pollination fails, pin flowers will usually be self-fertilized, and this may be important in very small populations.

I believe that it is justifiable to conclude that in both species pollination is efficient; that is, nearly all ovules will be fertilized, legitimately or illegitimately.

Genetics of heterostyly

Heterostyly in *Primula* is probably determined by a series of very closely linked genes, individually controlling pollen-grain size, anther height, style length, and possibly papilla length (Ernst, 1928, 1933). Crossing-over is extremely rare and such a complex behaves as a single gene; it is therefore convenient to use the notation of Bateson and Gregory (1905) who discovered for *P. sinensis* that thrum was dominant to pin, calling the genes S and s respectively. Cross-over combinations may be represented by using s with superscripts.

In natural populations, if there is no illegitimate fertilization, homozygotes SS will not occur, since pins must be ss. All thrums will be Ss. Legitimate fertilization will produce pins and thrums in equal numbers and this should be the normal population constitution.

In natural populations of *P. vulgaris*, pins are more numerous than thrums. I have counted about 80 normal populations in various parts of England. Of a

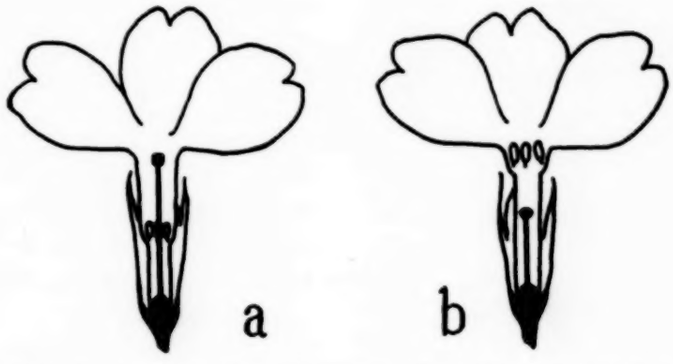

FIG. 1. *P. vulgaris*. Diagrams of half-flowers of the normal forms. (a) pin; (b) thrum.

total of 15,555 plants, 52.9% were pins; there was no significant heterogeneity between populations. In 20 population counts in Eastern England totalling 1639, Christy (1884) found 53.7% of pins. Haldane (1938) on the other hand found only 49.0% of pins among 2288 plants from 16 populations (mostly in Wales), but he counted plants so close together that error may have resulted from cloning.

This excess of pins in natural populations could be accounted for by a deviation from the backcross ratio of 1:1. Unfortunately, few data on backcross progeny are available for *P. vulgaris*. Another explanation is that some illegitimate pin fertilization occurs. It can be shown that 11% illegitimacy on pins, which is not an unlikely amount, would give an equilibrium population containing 52.9% of pins.

I prefer the second explanation, and consider that the breeding system in normal natural populations of *P. vulgaris* is one in which practically all thrum ovules are fertilized legitimately, while of the pin ovules about 90% are fertilized legitimately and about 10% illegitimately.

Homostyly

Linkage within the presumed gene-complex is not quite complete, and crossing-over does occur though very rarely. Ernst (1933) has described such crossovers in *P. viscosa*, and occasional plants of *P. vulgaris* have from time to time been reported to have anthers and stigma at the same level. Such forms have been called *homostyles*.

Crosby (1940) reported the occurrence in Somerset, England, of populations of *P. vulgaris* containing about 70% of a "long homostyle" form (fig. 2). The flowers of such a form have a gynaecium apparently identical, both morphologically and physiologically, with that of a pin.The corolla and androecium appear to be morphologically identical with those of a thrum and the pollen grains are of the same size and behave physiologically in the same way as thrum pollen grains do. The chromosome number of the homostyle is the same as that of the normal forms.

The crossover complex responsible for this homostyly may be represented by s′. It will be composed of the dominant thrum genes for androecium characters and the recessive pin genes for gynaecium characters. Long homostyly should therefore be dominant to pin and recessive to thrum, s′s being homostyle and Ss′ thrum. Experiment has shown this to be so.

Fig. 2. *P. vulgaris*. Diagram of half-flower of long homostyle.

Long homostyles should be fully self-fertile, since self-pollination is physiologically equivalent to pin × thrum, and therefore legitimate. They should also be fully fertile as male on pins and as female to thrums. These conclusions have also been experimentally confirmed.

Among open homostyle flowers (the qualifying "long" is omitted henceforth where there is no risk of ambiguity) the relative positions of stigma and anthers vary. Style length varies (just as it does in pins) and corolla tube length varies even more. Usually the stigma is in the base of the anthers, but it may be entirely below them, infrequently above but touching them, or very rarely above and quite clear of them. Except in the last case, the homostyle flower is almost bound to be self-pollinated. When the bud is about to open, the style has practically attained its final length but the

corolla tube has not. At this stage the stigma is always (except in deformed flowers) either above or among the anthers. As the flower opens, the corolla tube lengthens and the anthers are pulled over the stigma. Since, except in very cold or wet weather, the anthers dehisce at the same time, the stigma will almost always be self-pollinated before the flower is fully open and so before any thrum pollen can arrive on it. Even if self-pollination is avoided at this stage, the first insect visitor is almost bound to knock a large quantity of own pollen onto the stigma, and any thrum pollen arriving at the same time is likely to be heavily outnumbered.

In natural populations containing pins, thrums, and homostyles, the legitimate cross of homostyle × thrum would therefore be expected to be uncommon; it would occur only in those rare instances where the open flower has the stigma still above and clear of the anthers, or in flowers with deformed or empty anthers, or where anther dehiscence is delayed. The same considerations apply to the cross homostyle × homostyle and to the illegitimate homostyle × pin. The only important fertilizations affecting homostyles will then be pin × homostyle and homostyle selfed.

If this is true, plants of genotype Ss′ (phenotypically thrum) will be scarce naturally. Tests of thrums in wild populations have been made, but the results are not yet complete. It is known that Ss′ plants do occur, but their frequency seems to be low.

The almost complete exclusion of thrum pollen from homostyle ovules, combined with the presumed ability of homostyle pollen to compete for pin ovules on equal terms with thrum pollen with which it appears to be physiologically identical, gives homostyle pollen a decided advantage over thrum pollen. This would be expected to result in an increase in frequency of homostyles. Thrum pollen will carry either S or s in equal proportions, but the competitive disadvantage to s will be partly offset by the fact that a proportion of homostyle pollen grains also carry s; this proportion will vary according to the degree of homozygosity among homostyles, but will never be more than 50% and will usually be less. Both the normal forms should therefore decrease as homostyles increase, thrums more rapidly than pins. Preliminary counts from Somerset (Crosby, 1940) did show that where there were many homostyles, thrums were much less frequent than pins. A typical count in the wood where the homostyles were first discovered was 145 pins: 15 thrums: 468 homostyles.

Dynamics of Homostyle Populations

By making certain assumptions it is possible to construct a mathematical model of a population in which homostyles have arisen, and to trace the way in which they may be expected to increase in that population. But one complication needs to be considered first.

Since homostyles are self-fertilized, they can exist both as homozygotes s′s′ and as heterozygotes s′s. Mather and de Winton (1941) showed that homozygous thrum *P. sinensis* probably have a lower average viability than heterozygotes. Owing to much lower fertility of illegitimate thrum pollinations, such lower viability has not yet been demonstrated for *P. vulgaris;* if it exists and if it is a property of the thrum gene-complex itself, it might be expected that homostyle homozygotes would have a lower viability than heterozygotes since, if the crossover theory of the origin of homostyly is correct, the homostyle complex contains part of the thrum complex. It will be shown later that homozygotes are almost certainly less viable; there are grounds for believing (Crosby, 1948) that this low viability is due to a recessive pleiotropic effect of those genes responsible for the thrum-type androecium.

The possibility of low viability of homostyle homozygotes must therefore be taken into consideration in construc-

ting the model breeding system, but some assumption must first be made as to how this lowering of viability is expressed. It could appear as a reduction in the proportion of homozygotes which reach sexual maturity, as compared with heterozygotes pins and thrums, all forms being equally fertile; or it could appear as a decreased fertility of the homozygote, equal proportions of zygotes of all forms reaching maturity; or it could be a combination of both. There is no evidence at present which allows decision on this question, but for the purpose of this paper the mathematically more convenient assumption is made that all forms are equally fertile but a lower proportion of homostyle homozygotes reach maturity. The equations derived later can be applied to the opposite state of affairs by quite simple adjustments to the calculated proportions, and no qualitative difference arises in the conclusions reached.

The mathematical model of the breeding system in a pin-thrum-long homostyle population can now be constructed on the basis of the following assumptions, which have to be both simple and only approximate. The justifications for some of these assumptions have been discussed above.

i. The total ovule production per plant is the same for all genotypes.

ii. All ovules are fertilized.

iii. Total pollen production per plant is the same for thrums and both kinds of homostyle.

iv. The fertility relations of a homostyle are those to be expected from its thrum androecium and pin gynaecium.

v. Only legitimate fertilizations occur on thrums and homostyles, but the latter are always selfed.

vi. 10% of pin ovules are illegitimately fertilized. The remainder are fertilized at random by pollen of the other three genotypes and so from assumption iii by those genotypes in the proportions in which they occur among themselves.

TABLE 1. *Fertilization frequencies and zygotic ratios in a long-homostyle—pin—thrum population conforming to the assumptions made in the text. p q r s are the frequencies in the population of pins, thrums, heterozygous long homostyles and homozygous long homostyles respectively. v is the relative viability of homostyle homozygotes.*

Fertilization	Frequency	Proportions of viable offspring			
		ss	Ss	s's	s's'
Thrum × pin (Ss × ss)	q	$\frac{q}{2}$	$\frac{q}{2}$		
Pin selfed pin × pin (ss × ss)	$\frac{p}{10}$	$\frac{p}{10}$			
Pin × thrum (ss × Ss)	$\frac{9pq}{10(1-p)}$	$\frac{9pq}{20(1-p)}$	$\frac{9pq}{20(1-p)}$		
Pin × l.h. (ss × s's)	$\frac{9pr}{10(1-p)}$	$\frac{9pr}{20(1-p)}$		$\frac{9pr}{20(1-p)}$	
Pin × l.h. (ss × s's')	$\frac{9ps}{10(1-p)}$			$\frac{9ps}{10(1-p)}$	
L.h. selfed (s's × s's)	r	$\frac{r}{4}$		$\frac{r}{2}$	$\frac{rv}{4}$
L.h. selfed (s's' × s's')	s				vs

vii. The zygotic ratios from any type of fertilization are those expected theoretically.

viii. Seeds containing embryos of constitution Ss, ss, or s's are equally viable, viability being defined as the ability of the seed under normal conditions to germinate and produce mature seed-bearing plants. The relative viability (hereafter referred to simply as the viability) of s's' zygotes is that proportion of them which give mature seed-bearing plants divided by the corresponding proportion for the other types.

ix. The plants of any one generation come from a random selection of the viable seed of the preceding one.

x. There is no overlap between generations. This is not true, but nothing is known at present of the degree of overlap; a contrary assumption would complicate the mathematics without having any qualitative effect on the result.

Precise correctness is not claimed for any of these assumptions, but they give a good working basis for an algebraic treatment of the breeding system. All are capable of being true, and the population model constructed on their basis is a possible one. How far the resulting conclusions may be applied to natural populations does depend upon the accuracy of the assumptions, and conversely, the constructed model will be judged for accuracy of representation of the natural situation (though the judgment will not be rigorous) by how far the conclusions drawn from it correspond with observable facts.

Consider a population consisting of pins (ss), thrums (Ss), heterozygous long homostyles (s's), and homozygous long homostyles (s's'), in the respective proportions $p\,q\,r$ and s, where $p+q+r+s=1$. The viability of homozygous homostyles is v.

Then the possible fertilizations will occur with the frequencies shown in table 1, which also shows the ratios of viable zygotes resulting from them. By summing the vertical columns of progeny proportion in that table we obtain four values which on dividing by their total give $p_1\,q_1\,r_1$ and s_1, the respective proportions in the next generation. The division is necessary because that total is less than unity when v is less than 1, whereas $p_1+q_1+r_1+s_1=1$.

TABLE 2. *The proportions ($p_1\,q_1\,r_1\,s_1$) of the four types in any one generation, calculated from those ($p\,q\,r\,s$) of the preceding one. v is the relative viability of homostyle homozygotes.*

Pins	$p_1=\dfrac{p(2-2p-q+4r)+10q+5r}{5(1-p)\{4-(1-v)(r+4s)\}}$
Thrums	$q_1=\dfrac{q(10-p)}{5(1-p)\{4-(1-v)(r+4s)\}}$
Heterozygous homostyles	$r_1=\dfrac{18ps+r(10-p)}{5(1-p)\{4-(1-v)(r+4s)\}}$
Homozygous homostyles	$s_1=\dfrac{v(r+4s)}{4-(1-v)(r+4s)}$

Expressions for $p_1\,q_1\,r_1$ and s_1 derived from $p\,q\,r$ and s are given in table 2, and by their use it is easy, given the proportions of the four genotypes in one generation, to calculate them for the next for any value of v. From that generation the following one may be calculated, and so on. Curves showing population change can then be drawn, and from these the fate of a homostyle gene-complex s' arising in a normal population

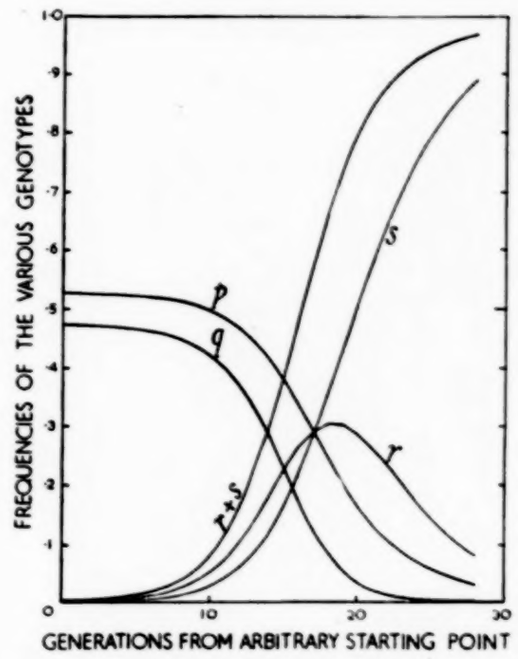

FIG. 3. Curves of homostyle population change for complete viability of homostyle homozygote ($v=1$). p = pins, q = thrums, r = heterozygous homostyles, s = homozygous homostyles.

of pins and thrums can be predicted, the path by which the population will shift to a new equilibrium or revert to the old one can be traced, and the final equilibrium constitution determined.

Curves of population change

Such calculated curves of population change are shown in figures 3 and 4. In each case the starting point, generation 0, is an equilibrium pin-thrum population (52.7% pin) to which has been added one heterozygous homostyle for every 499 normal plants.

In calculating figure 3 the homostyle homozygotes have been supposed to be fully viable. It can be seen that homostyles increase in the population, though slowly at first. After 5 generations there are just over 1% of them, two-thirds being heterozygous. They then increase more rapidly, reaching 8% after 10 generations, while at 16 generations half the population is homostyled. Both pins and thrums decrease, the latter more rapidly, though this is not so obvious in the first part of the graph because of the initial separation of the pin and thrum curves; after 16 generations thrums have fallen to 18% and pins only to 33.5%.

After 16 generations the rate of change slows down and homostyle homozygotes, which have always been increasing the more rapidly, become more frequent than heterozygotes. The latter reach a maximum value of 30.5% at generation 18 and then decline. Thrums practically disappear soon after this, being only 0.2% at generation 25. By generation 28 the population contains 97% of homostyles and 92% of these are homozygous. The endpoint is complete homostyly.

Thus when the homozygote is fully viable final equilibrium is a population consisting entirely of homozygous homostyles. The curves approach this equilibrium more and more slowly, but in natural populations it would be reached quickly as a result of fortuitous elimination of the s-complex when its frequency is very low.

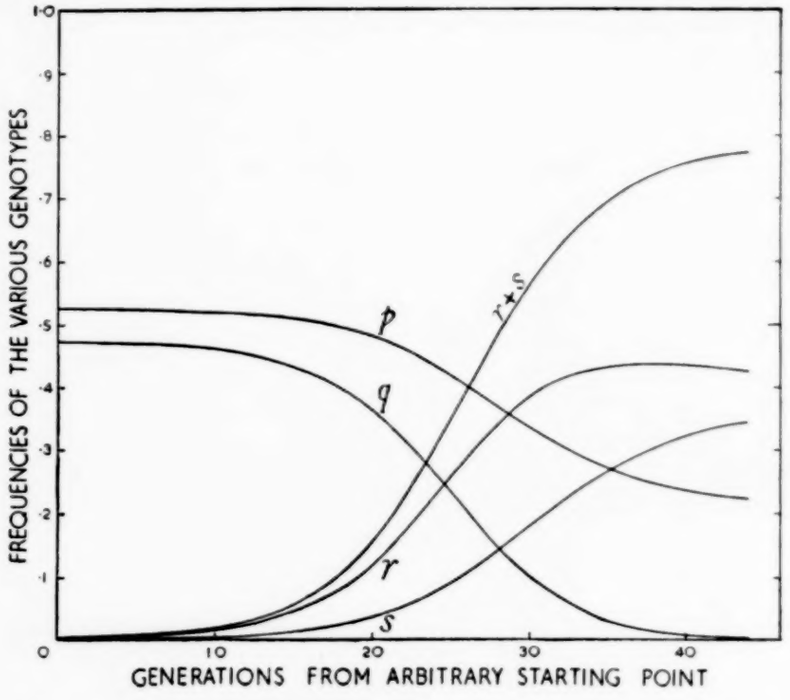

FIG. 4. Curves of homostyle population change when the relative viability, v, of the homostyle homozygote is 0.65. p = pins, q = thrums, r = heterozygous homostyles, s = homozygous homostyles.

The final equilibrium can be deduced from the equations, for then

$$s = s_1 = \frac{r + 4s}{4},$$

and so $r = 0$. But $r = 0$ only when $s = 0$ or when $s = 1$. That is, when homostyle homozygotes are fully viable, equilibrium either contains no homostyles (a normal pin-thrum population) or else consists entirely of homozygous homostyles.

The more rapid decrease and earlier extinction of thrums was expected and results from the disadvantage which thrum pollen has in competition. It does not arise from any advantage which the pin genotype might be expected to have as a result of its 10% illegitimacy. Corresponding population curves calculated on the assumption of no illegitimacy also show a more rapid decrease of thrums. Indeed, if instead of actual frequencies of pins and thrums, fractions of their starting frequencies are plotted, the two sets of curves (10% and no illegitimacy) are almost identical for most of their length. The two homostyle curves as they stand are practically the same for the two cases. The introduction of pin illegitimacy into the model of the breeding system has no qualitative effect either in this example or in the one now to be discussed.

With lower values of homozygote viability, a qualitatively different result may be obtained. Fig 4 shows the curves calculated for $v = 0.65$. Here, change is much slower, and homostyles do not reach 1% until generation 8. They reach 8% in 17 generations and 50% is not exceeded until generation 29, when 70% of them are still heterozygotes. Although the homozygotes always increase relatively more rapidly than heterozygotes, they never outnumber them at this value of the viability.

The more rapid decline of thrums is much more obvious. They drop to 10% in 30 generations, while pins have only fallen to 33.5%. After 44 generations thrums are practically extinct at 0.2%, but pins show no signs of extinction. Contrary to the case where $v = 1$, pins appear to be approaching a steady value of about 20%. Homostyles also are approaching a steady value at about 80%; heterozygotes reach a maximum value of 43.6% at generation 37 and then decline slightly, but they will still number a little over half of the total homostyles at equilibrium.

Thus when the viability of the homozygous homostyles is only 0.65, population change does not proceed to complete homostyly. Thrums are entirely eliminated, as before, but the final equilibrium should contain 20% of pins and only 80% of homostyles.

The calculation of this equilibrium is not so simple as it was with the previous example, for the equation

$$s = s_1 = \frac{v(r + 4s)}{4 - (1 - v)(r + 4s)}$$

has more than one solution except when $v = 1$ or 0.

It is sufficient to solve any two of the equations $p_1 = p$, $r_1 = r$, $s_1 = s$, for it can be shown that for any equilibrium value, $q = 0$ when $r > 0$ and $v > 0$.

Consider any population. Then from table 2

$$\frac{q_1}{r_1} = \frac{q(10 - p)}{18ps + r(10 - p)} < \frac{q}{r}$$

except when $ps = 0$ or $q = 0$.

When $ps = 0$, either $p = 0$ or $s = 0$. If $p = 0$, then equilibrium is only possible when $q = 0$ (since thrums cannot reproduce in the absence of pins). When $s = 0$, either $r = 0$ and the population consists of pins and thrums in equilibrium, or $v = 0$.

Therefore $\frac{q_1}{r_1} < \frac{q}{r}$, and the population is not in equilibrium, unless one of the three values q r or v is zero. Hence in any population containing homostyles with homozygote viability greater than

zero, thrums will disappear before equilibrium is reached.

A general algebraic expression can now be derived giving the constitutions of equilibrium populations for any positive value of v. The derivation is too lengthy to give in full, but a brief indication of the method used is given.

At equilibrium $q = 0$, and so

$$p = p_1 = \frac{2p(1 - p + 2r) + 5r}{5(1 - p)\{4 - (1 - v)(r + 4s)\}}$$

s may be eliminated by substituting $1 - p - r$, and manipulation may be simplified by putting a for $p + v - pv$. This then gives

$$r = \frac{2p(1 - p)(10a - 1)}{(5 - 11p + 15pa)}. \qquad (1)$$

Also, at equilibrium

$$r = r_1 = \frac{18ps + r(10 - p)}{5(1 - p)\{4 - (1 - v)(r + 4s)\}}.$$

Eliminating s and substituting for $p + v - pv$, as before, and now eliminating r by use of equation (1), we are left with an equation in terms of p and a. This may be simplified first to the form $ba^2 + ca + d = 0$, where b c and d contain only p. a may now be replaced by $p + v - pv$, and this eventually gives the equation

$$\begin{aligned} &55(1 - v)^2p^4 + (79 - 249v + 170v^2)p^3 \\ &\quad - (126 - 867v + 705v^2)p^2 \\ &\quad - (43 + 628v - 680v^2)p \\ &\quad + (35 + 120v - 200v^2) = 0. \end{aligned}$$

This may be transformed from an equation of the form $fp^4 + gp^3 - hp^2 - ip + j = 0$ into one of the form

$$p = \frac{j}{i + hp - gp^2 - fp^3},$$

from which for any value of v the corresponding value of p may be rapidly determined by iteration. r is easily calculated from equation (1), and s from the equation $p + r + s = 1$.

Figure 5 shows the equilibrium constitutions for different values of v (it excludes the metastable equilibria of $r + s = 0$ and $s = 1$, which are possible for all values of v).

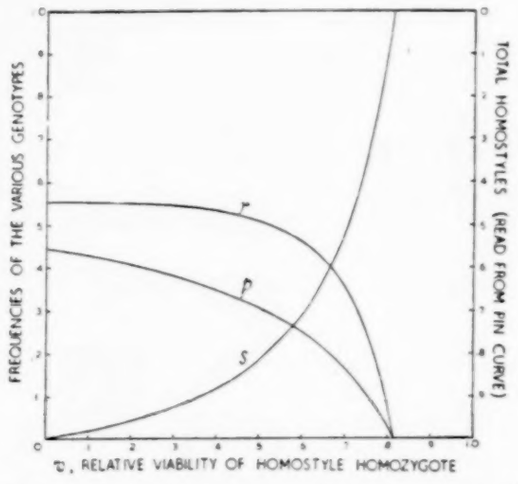

FIG. 5. Constitutions of equilibrium populations with different values of the relative viability, v, of homostyle homozygotes. p = pins, q = thrums, r = heterozygous homostyles, s = homozygous homostyles.

There are two main points of interest. Firstly, there should be at least 55.5% of homostyles at equilibrium whenever $v > 0$. Hence when $v > 0$ homostyly should increase in frequency in any population in which it arises. However, where v is low, initial increase of homostyles would be so slow that their frequency would be much more subject to random fluctuation than to selection. For complete inviability of homozygotes the curves do not apply, since they have been calculated on the assumption that $v > 0$. Actually, when $v = 0$ there is a continuum of equilibria conforming to the equation $p = \frac{10 - 5r}{19 - 5r}$ from $r = 0$ to $r = 0.555$. Fig. 5 gives only the $q = 0$ endpoint of this continuum.

The second striking point is that not only will the homostyle homozygotes increase in frequency from very nearly zero for any but the very lowest positive value of v, but when v is greater than 0.815 this increase continues until they

reach 100%. That is, the homozygote may be selected to replace completely the other three genotypes even when its viability is lower than theirs. The implications of this will be dealt with in the discussion.

Under natural conditions, random fluctuations will cause populations to diverge from the calculated curves. Calculation will show that such divergent populations are expected to approach the curves closely again in one generation, except possibly when thrum frequency is very low.

Results of Population Counts

The homostyles were first recognized in samples of primroses sent to me from Sparkford in 1940. Owing to the war I was unable to go there myself until 1946. Professor R. A. Fisher kindly made available to me unpublished data of population counts which he made around Sparkford in 1942, 1943, and 1944. Part of these data have been used in the preparation of figures 6 and 8 where it was desirable to supplement my own data. Other and later data of Professor Fisher's have not been used in this account. Dr. D. G. Catcheside secured local co-operation in obtaining samples, and visited the area himself in 1945. He also kindly passed his data to me, and they have been incorporated in this account, together with some genetical data which will be referred to later.

I carried out extensive counting in 1946, 1947, and 1948, and found that the homostyles occurred over an area at least 15 miles across. In 1944 I found a second homostyle area of about the same size on the Chiltern Hills, in Buckinghamshire, and made counts there that year, in 1945, 1946, and 1948.

The results from population counts are set out in the form of graph and maps. In counting a population my aim was to obtain a random sample, numbering

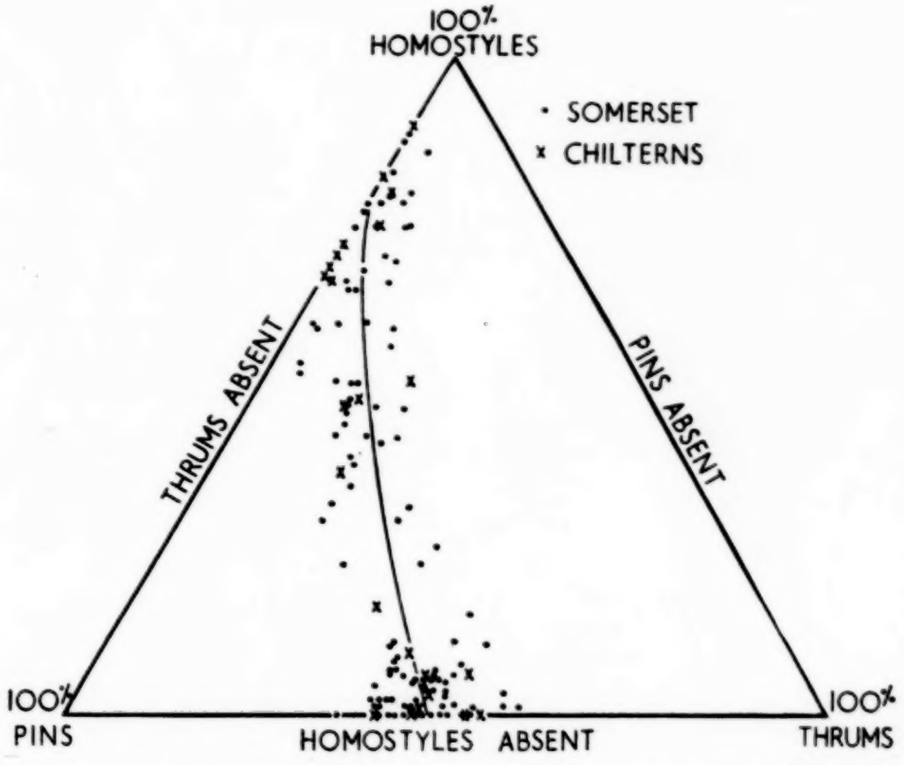

Fig. 6. The constitutions of natural populations of *P. vulgaris* in the homostyle areas, with the theoretical curve of population change for $v = 0.65$ added. Sample size greater than 90.

if possible about 100, by rapidly picking single flowers from plants taken as evenly as possible over the area of counting. Care was taken not to sample plants too close together, to avoid error due to cloning. In small populations it was sometimes necessary to count most plants in order to get 100, but care was always taken to try to avoid picking two flowers from one clone. In many populations so large a count was not possible.

Large populations were usually counted in sections, each section sample if possible being about 100 plants. This was necessary because it was found that such populations tended to be heterogeneous; that is, different parts of an apparently single population were sometimes at significantly different stages of the presumed evolution to homostyly. (Such intrapopulation heterogeneity implies a very short breeding range of the individual plant; this will be the subject of a later paper.)

Population constitutions

The population proportions of counts of more than 90 from both homostyle areas are given graphically in figure 6. Where a population was counted in sections each sectional count is given individually. Where a population was counted in more than one year the latest count of over 90 has been used.

The use of a triangular chart (suggested to me by Prof. R. A. Fisher) enables each count to be represented by a single point. Three variables totalling unity are involved (the two kinds of homostyle cannot at present be distinguished by inspection), and they can be represented by the perpendiculars from a point inside an equilateral triangle to its three sides. That is, in figure 6 the frequency of pins represented by any point is the perpendicular distance of that point from the "pins absent" side of the triangle, and those of thrums and homostyles correspondingly by the distances from the appropriate sides.

It is clear from the figure that the population constitutions show considerable regularity. High homostyle populations, as was predicted, have far fewer thrums than pins, and from 12 populations of this size (and from many more smaller ones) thrums are entirely missing. No population of this size lacks pins; the largest entirely homostyle count was 19, and most were much smaller. The trend of population change seems to be to an endpoint of rather less than 80% homostyly. Reference to figure 5 shows that the model system would reach the same endpoint when $v =$ about 0.65. The curve of population change for this value of homozygote viability, representing the three curves for pins, thrums, and total homostyles of figure 4, is added to figure 6. It can be seen that there is good agreement between the population data and the curve throughout its length.

Most of the points are concentrated near the beginning and end of the curve of change, as would be expected since these should be the regions of slowest change. This effect would be greater in the figure were it not that in deciding where to count populations I often aimed at districts where change might be expected to be most rapid, so there are probably more points in the central region than there would be with a random choice of populations.

The regularity of the points in the graph does strongly suggest that they represent populations at different stages of the same evolutionary process, and it also appears that there may be no essential difference in their dynamics between the Somerset and Chiltern populations.

Distribution of homostyly in the two areas

The distribution of the homostyles is shown in the maps, figures 7 and 8. They show the size class of the population count, and the range of constitutions within which it falls. Four size

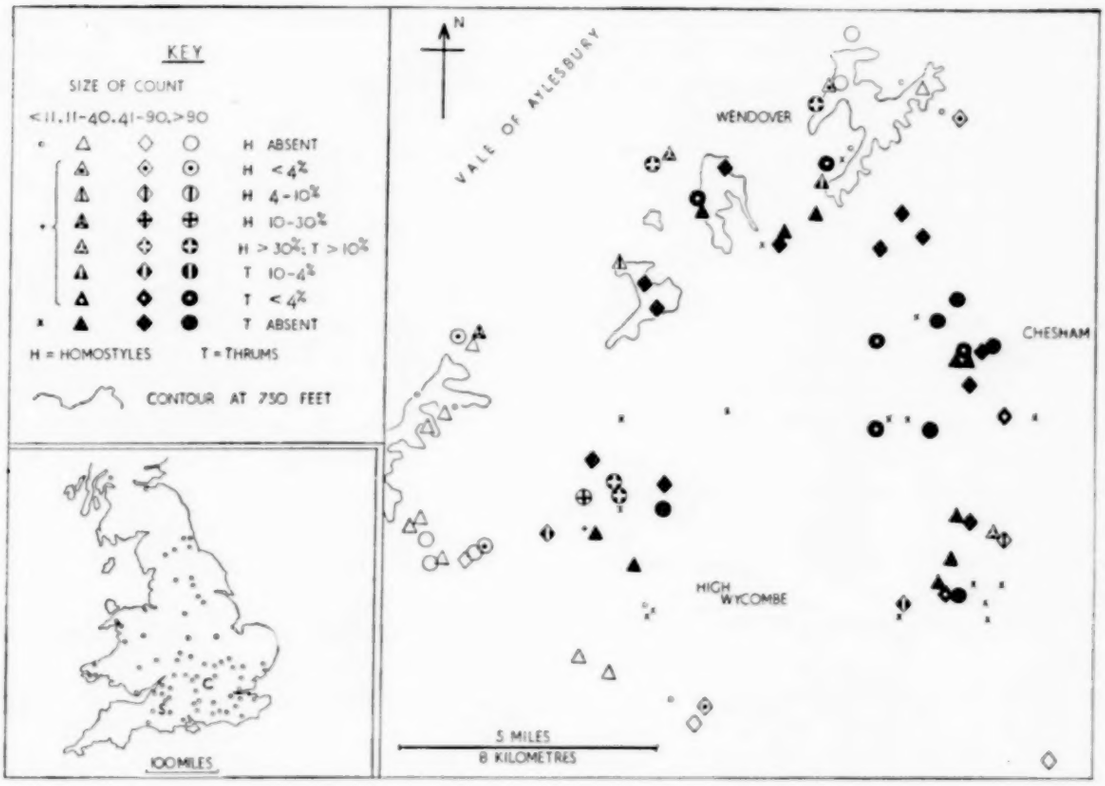

FIG. 7. Distribution of long homostyle *P. vulgaris* in the Chilterns. Inset: map showing location of the two areas, and of populations which have been examined and found to contain no homostyles.

groups are used, but the smallest counts are often omitted where the map is crowded and they would add no important information. No count containing pins only has been included. For the three smallest groups the count size is a rough indication of the size of the population.

For indicating the constitution, the supposed course of evolutionary change has been divided into six stages, so chosen that a population would be expected to take about the same time to pass through each. For the beginning, homostyle frequency is the criterion of progress; for the end, thrum frequency is used.

Where populations were counted in sections, the sectional counts cannot be given individually on the maps; they fall as a rule within the same constitution class. Sometimes distinct populations were too close together to be separable. In both cases the separate data have been compounded and are represented by a single symbol which gives the size group of the grand total. Where slightly displacing the symbols on the map allows two neighbouring populations to be separately represented, this is done, especially where their constitutions fall into different classes. The S.W. corner of the Somerset map presents particular difficulties, for so many counts have been made there; the compounding is done so as to present as true a picture as possible of homostyle distribution in that district. Where a population was counted in more than one year the largest count is usually preferred, except that of counts over 90 the latest one has been used.

The maps support the graph in providing strong evidence of some evolutionary change. In both cases homostyly decreases in frequency as one passes outwards from the center. This is most obvious in the Chilterns (fig. 7) where the homostyle area is approximately iso-

diametric and the frequency change fairly regular with a large central region of thrum absence. Unfortunately primroses have become scarce on the Chilterns within the last 50 years, due to intensive picking and uprooting. They are common nowhere except in the S.W., where homostyles are infrequent, and no populations comparable with the larger Somerset ones were ever found. As a result, there are too few counts for a wholly satisfactory picture to be obtained of the distribution of homostyly on the Chilterns. The scarcity of counts from the central region is partly due to scarcity of primroses near High Wycombe and partly to concentration of counting round the edges of the area.

A further complication in the Chilterns area is found in the N.E., where there appears to be an occasional modification which makes it difficult in a few instances to distinguish thrums and long homostyles. This was discovered in 1948 and affects very few of the populations. The situation is interesting and is being investigated.

Homostyles probably do not occur in the Chilterns area outside the range already counted. Both to the N.W. and to the E. it might be thought likely that they extend into uncounted parts, but the limit of the known homostyle range is probably the local boundary of the species; I could not find primroses in the vale, and they almost certainly do not occur naturally in the built-up district between Chesham and London, except possibly in some private parklands which have not yet been investigated. Homostyles can still increase in frequency in the N.W. boundary populations, but on the E. side most of the boundary populations have reached the presumed final

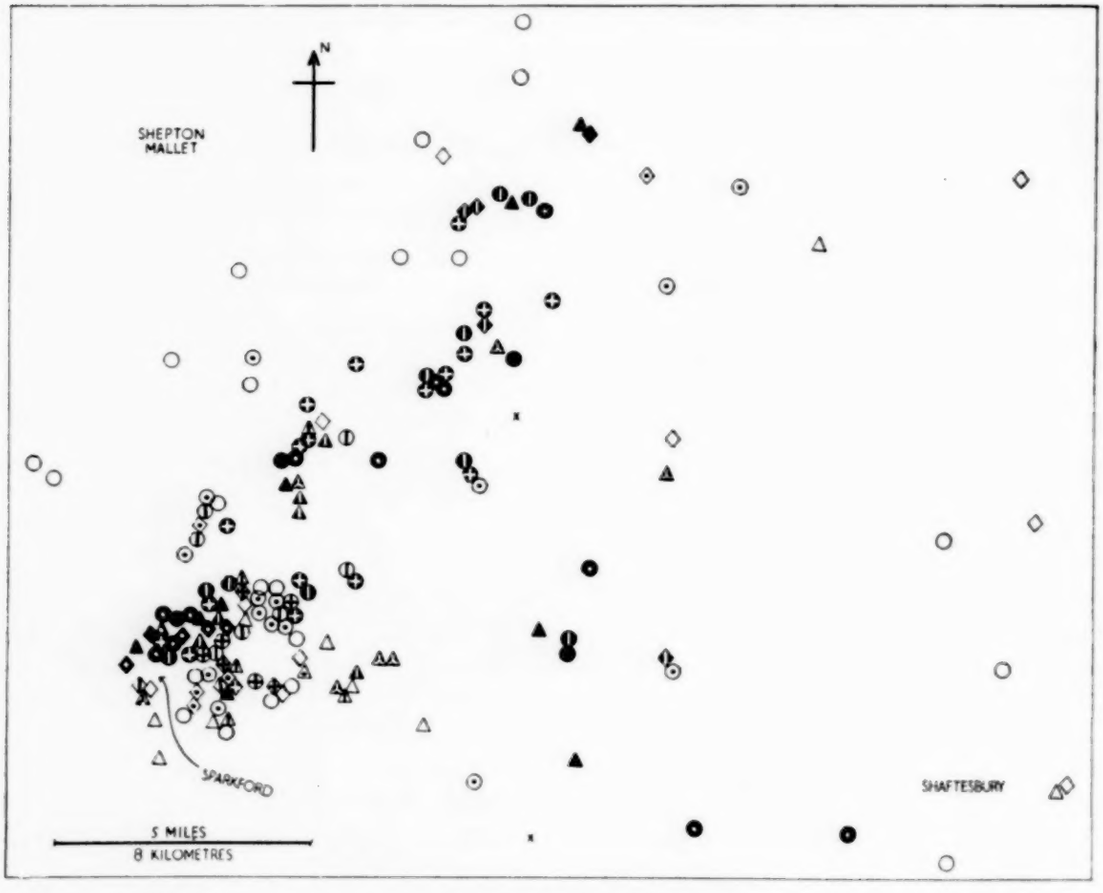

FIG. 8. Distribution of long homostyle *P. vulgaris* in the Somerset area (which extends into Dorset and Wiltshire). Key as for figure 7.

equilibrium, with complete elimination of thrums.

The Somerset map (fig. 8) provides a much more satisfactory picture of a homostyle area. The apparent lack of a boundary to the S.W. is due to absence of primroses from the clay soils S.W. of Sparkford, so here also homostyle and primrose boundaries coincide. The S.E. part of the area has not yet been fully investigated, and it is not known how far in that direction homostyles extend. Elsewhere an adequate number of large counts have been made. The uneven distribution of counts shown in the map is due partly to concentration of counting near Sparkford and partly to local scarcities of primroses, particularly on high ground in the eastern half of the area.

Less evolutionary progress has been made in the center of the area, as compared with the Chilterns; few Somerset populations, except very small ones, are without thrums. There is no continuous region of high homostyle concentration, and this suggests that from an initial center of homostyly secondary centers may have arisen by very rare distant transport of pollen or seed. The system which is used in the maps of dividing the course of population change into a few sections largely obliterates minor irregularities in the homostyle gradient; an overlying regularity in the decline in homostyle concentration outwards from each center can then be seen. The minor irregularities which the detailed figures would show in no way invalidate the inference from the maps of a steady outward migration of homostyles, for since heterogeneity can exist within populations, a completely regular gradient of homostyle concentration is not to be expected as one passes outwards from population to population.

Homostyly may be more recent in Somerset than in the Chilterns; further evolution should lead to spreading of the centers of high homostyle frequency and their eventual fusion, giving a large thrumless area as in the Chilterns. The possibility must also be considered that the much smaller size and greater separation of the Chiltern populations may have led fortuitously to more rapid extinction of thrums there.

The distribution of homostyly in the two areas provides strong but not conclusive evidence that an evolutionary change from heterostyly to partial homostyly is in progress. The only other possibility is of a change in the reverse direction, for some change must be occurring; there would be much less regularity in distribution if there were a whole series of equilibria represented by the different populations.

It is difficult to conceive the change occurring in this reverse direction. This difficulty of imagining the homostyle areas as contracting, as relics of a once large area of homostyles, is certainly partly subjective, but it would be a remarkable coincidence if the populations followed the theoretical course of population change, but in the opposite direction to that predicted. Perhaps the best argument is that change to heterostyly would imply an original condition of pins and long homostyles in *P. vulgaris,* a condition unknown in any other species of *Primula.* It seems almost certain that heterostyly is a primitive condition of the genus. Not only is it widely distributed within it, but it occurs in typical form in a few other genera of the *Primulaceae;* Kuhn (1867) gives *Dionysia, Gregoria,* and *Hottonia.*

Homozygote viability

The close agreement between the population counts and the $v = 0.65$ curve is good evidence that the general basis on which the population model was constructed was correct, but certain other tests are possible and necessary. The most obvious of these is of homozygote viability. Direct tests on viability have not yet been made, and it is doubtful how far results obtained under garden conditions can legitimately be applied to

wild populations. Some indirect evidence can be obtained by determination of the degree of homozygosity among wild homostyles. Comparison of figs. 3 and 4 shows that for different values of v populations with about 75% of homostyles will have quite different proportions of homozygotes. For $v = 1$, 60% of homostyles are homozygous, for $v = 0.65$, 42% are. This proportion can be determined genetically. A large series of test-cross progeny has not yet flowered, but some results of experiments by Dr. D. G. Catcheside may be given. Unfortunately, most of his families were too small for the results to be more than suggestive (Crosby, 1948).

From 3 populations containing 78%, 79%, and 66% of homostyles, of 59 homostyles tested not more than 29 could have been homozygous, and the small size of non-segregating families makes the probable number lower. The most likely value was that 20% of the homostyles were homozygous. From a population containing 33% of homostyles, 13 were tested and 3 (23%) were homozygous. The figures are certainly liable to considerable error, but they do suggest that the degree of homozygosity is much lower than would be expected with complete viability.

Direct detection of change

The real criterion of population change must be its direct detection by successive counting of individual populations. Attempts have been made to do this, but the difficulties are considerable (Crosby, 1948). Large errors may arise from several causes. Intrapopulation heterogeneity will have an effect if the areas of counting are not precisely the same on each occasion and if sampling is uneven; these are controllable, but change in population density, which experience has shown may occasionally be considerable, is not, and its effect is quite as great. There is a little evidence that the four genotypes may have slightly different flowering periods, and this would cause error if counts in successive years are made at different stages of the flowering season. Random deviation in one generation from the constitution expected from the previous one may be greater than the change expected over several generations, unless very large counts are made; but these would add to the difficulties due to heterogeneity. Change is almost certainly not quick enough for it to have been detected in my own counts.

A double attack is being made on the problem. Small plots have been marked out and all plants within them charted: they will be followed from year to year, and detailed information will be obtained on population change, seed production, etc. In addition, as many accurately delimited populations as possible will be counted in successive years. If the number of these is large enough the errors may be sufficiently minimised to give a useful result in several years time. But rapid direct evidence of population change cannot be expected.

The general distribution of primrose homostyly

The inset map of figure 7 shows the two homostyle areas and also the British localities where population counts of *P. vulgaris* have been made and homostyles not found. This is mainly based on my own counts or on counts made by various people for me, but it includes published data of Darwin (1877), Christy (1884), and Haldane (1938). It seems clear that for most of England there is no comparable homostyle area; counting of regions so far uninvestigated will continue. There is no evidence in the literature of areas of homostyle primroses elsewhere than in Britain, though solitary plants have occasionally been reported. The two areas described here are thus the only ones known where homostyle primroses are frequent.

Discussion

Although the evidence presented here is largely circumstantial and further re-

sults are necessary, there are good grounds for believing that in broad outline the primrose populations in the two areas described do conform to the constructed model of a breeding system under conditions of partial inviability of homostyle homozygotes. This is deemed sufficient justification for basing the following discussion primarily on the model system, and for considering it valid to suppose that the conclusions reached may be applicable to natural populations.

Where homostyle homozygotes are less viable than the other three genotypes, increase in their frequency means a lowering of the average fitness of the species expressed as a decrease in the production of viable seed per generation, whether resulting from inviable zygotes or from reduced adult fertility. If the environment and the strength of competition from other species do not change, this will lead to a reduction in or reversal of the rate of increase of an expanding species, to a decrease in numbers of a previously static species, or to a greater rate of decrease in a declining one. In each case the s′ gene-complex is unfavourable to the species; on general selection theory it would be expected to decrease in frequency as a result of selection against it. But here it is clear that for a period at least this complex will increase from very nearly zero whatever the relative viability of its homozygote. That is, initially s′ has a selective advantage, although this falls off as its degree of homozygosity rises. If $v >$ 0.815, s′ maintains its advantage until both S and s are eliminated and 100% homostyly is reached. But when $v <$ 0.815 its advantage falls to zero and equilibrium is reached while s complexes but not S are still present. The latter situation seems to obtain in nature, but there is no difference in principle between the two cases.

The anomaly arises because the effect of s′ is different at different stages of the life cycle, while these stages also differ in their degree of interspecific competition. In phanerogams, interspecific competition at the gametophyte stage takes the form of competition between pollen grains for ovules; it will occur between those species which hybridize in natural conditions, whether the hybrid zygote is viable or not. The model system excluded the possibility of such interspecific competition, since no foreign pollen was admitted. This probably applies equally to the studied populations of *P. vulgaris;* the only possible competitor is *P. veris,* which does not come into flower until *P. vulgaris* has nearly finished flowering, and which does not occur sufficiently close to many of the populations for contamination to be important. It is therefore assumed that in the system under consideration interspecific competition at the gametophyte stage may be neglected; intraspecific competition may however be strong. Interspecific and intraspecific competition occur together in the sporophyte generation, and it is at this stage only that the s′-complex has an effect on species fitness.

The selection of a gene reducing species fitness (not to be confused with purely fortuitous increase which is possible in small populations) must mean that it possesses an advantage in gametophytic intraspecific competition which outweighs its disadvantage in sporophytic competition. This is the situation in homostyle primroses, although paradoxically the gametophytic advantage is sporophytically determined, since it depends on flower structure. Because of their almost complete monopoly of homostyle stigmas, pollen grains from homostyles have an advantage over those from thrums. This leads to an advantage of s′ over S and, to a lesser degree, over s, as has been pointed out earlier. There wil' therefore be an increase in frequency (selection) of s′ between sporogenesis and fertilization.

In the sporophyte generation the frequency of s′ will fall, either because of its elimination in inviable s′s′ zygotes, or because lower fertility of s′s′ plants re-

sults in a lower production of s′ spores, or for both reasons. Decrease in frequency (contraselection) of s′ between fertilization and sporogenesis will be greater the greater the frequency of s′s′ zygotes. That is, contraselection will rise with increasing homostyly.

The dynamics of the situation may thus be reduced to the opposition between gametophytic selection and sporophytic contraselection, and equilibrium is the point at which they balance. When $v >$ 0.815 contraselection never becomes large enough to balance selection, and s′ reaches 100%. When $v < 0.815$ a point is reached short of complete homostyly when selection and contraselection balance, but not before S is eliminated; s′ always has a selective advantage over S, and in equilibrium populations only s and s′ are involved.

The application of the model system to *P. vulgaris* depends very much on the validity of assumption ii, that all ovules are fertilized. If it were false, it might be expected that homostyles because of their self-pollination would have a greater seed output which might compensate partly or entirely for inviability of some of their seed. This point is highly critical, and it is hoped to obtain seed production data as soon as possible. This will be difficult; such data are easily obtained from garden plants, but their application to wild populations is of very doubtful validity. Meanwhile it can be said that if pin and thrum pollination is fully efficient, as preliminary data suggest, no greater seed output from homostyles is to be expected from their self-pollination. Further, it can be shown theoretically (Crosby, 1948) that if compensation for inviable seed were complete the only possible homostyle-containing equilibrium for any positive value of v would be 100% of homozygotes. If compensation is not complete, then there is a deficiency in the total production of viable seed by the species, which has therefore a lower fitness.

Any difference which may exist between the selective activity and the effect on the average survival value, in Fisher's (1941) sense of these terms, arises because the former refers to both generations and may be different in direction as well as in magnitude in each, while survival value is considered only in relation to the sporophyte generation. Different alleles will usually have little if any difference in advantage at the gametophyte stage except where the breeding system is affected. As a result the selective advantage of a gene substitution will usually be its selective advantage in the sporophyte generation and will correspond to its effect on survival value of the population. It is when there is selection of or against a gene away from the sporophyte generation that the divergence between selective activity and effect on average survival value becomes important, and this may happen whenever the gene change affects the breeding system. Under such circumstances the selection of a gene within the species may be to the disadvantage of the species, leading to reduction in numbers or even to extinction, both of the species and of the gene.

But here a further consideration arises. "Equilibrium" is final only in a relative sense. Selection of genes increasing the viability of s′s′ is almost sure to occur, and may be rapid enough to save the species from extinction. It would involve at the same time a shift of the equilibrium and eventual elimination of pins. The result might be a completely homostyled species as "fit" as it was in the original condition, but lacking the genetic plasticity of an outbreeding species.

This may have happened to certain species of *Primula*, which are homostyled. If heterostyly is primitive in the genus, as seems most likely, these homostyled species will be so secondarily. It is tempting to suppose that *P. vulgaris* is in a condition through which these species have passed, and that it will eventually become completely homo-

styled. No other *Primula* species shows a like condition, but the Gentianaceous *Menyanthes trifoliata,* which is normally dimorphic heterostyled, is homostyled in West Greenland (Knuth, 1909).

If selection of modifiers of an increasing unfavourable gene is not rapid enough, extinction seems likely. One can only speculate how rare or how frequent this may have been in the past, but it provides a possible explanation for those cases where a species seems to have suffered rapid extinction. The selection of unfavourable genes may sometimes have been an important factor in evolution.

In the limited space available it has been impossible to deal with many of the problems which homostyly has raised. It is intended to produce a fuller discussion in a later paper.

Summary

Primula vulgaris is a dimorphic heterostyled species. Because of self-incompatibility reactions the breeding system is essentially one of outcrossing between "pins" (ss) and "thrums" (Ss), which differ reciprocally in gynaecium and androecium arrangement. Pins are slightly the more frequent in natural populations.

In two areas in England a homostyle form (s's' or s's) is abundant. Having the gynaecium of a pin and the androecium of a thrum, it is self-fertile and almost always self-pollinated. Self-pollination excludes the compatible thrum pollen from homostyle stigmas, giving the s' complex an advantage among pollen grains. Heterozygotes are probably as viable as pins or thrums; homozygotes may be less viable.

Theoretical treatment of a breeding system containing all three phenotypes shows that homostyles should increase in frequency in any population in which they arise, unless homozygotes are completely inviable. Thrums should disappear entirely; so should pins if the relative viability of homostyle homozygotes is greater than 0.815, only the s' complex remaining in the population. When the homozygote is less viable, an equilibrium of pins and homostyles should be reached.

When homostyle homozygotes are less viable than the other forms, s' is harmful to the species. Its increase in frequency (apart from chance fluctuations) is then the selection of an unfavourable gene-complex. This can happen because selection against the complex during the sporophyte generation, when s' lowers the fitness of the species for interspecific competition, is opposed by selection of homostyle pollen during the gametophyte generation, when there is no interspecific competition.

Population counts in the two homostyle areas suggest that homostyles are increasing in the way predicted, and that final equilibrium populations will contain 80% of homostyles, 20% of pins, and no thrums. This would be expected with homozygote relative viability of 0.65, and population constitutions approximate to the curve of population change drawn for that value of the viability.

Selection of genes increasing homozygote viability can be expected to shift the equilibrium towards complete homostyly. *P. vulgaris* would then become a homostyled species. Homostyled species in normally heterostyled groups such as *Primula* may have become homostyled in this way. Similar occurrences may even have led to the extinction of a species, when selection for increased viability has not been rapid enough to prevent it.

Acknowledgments

I am especially grateful to Dr. D. G. Catcheside, not only for guidance and encouragement, but also for the maintenance of my plants during the war and for the results of population counts and breeding experiments during the same period. My thanks are also due to Professor R. A. Fisher for his interest, suggestions, and the use of his data; to Mr. R. C. Bullock of Sexey's School, Bruton, whose local knowledge has been of great value to me on my visits to Somerset;

to Mr. D. W. P. Greenham, who first found the homostyles; to many friends who have sent me counts or samples of primroses from various districts; to Dr. D. H. Valentine for criticism of the manuscript of this paper; and to the Council of the Durham Colleges, who contributed substantially towards the cost of the field work in 1947 and 1948.

Prior to 1947 these investigations were carried out at the Botany School, Cambridge, England. This account is a rewritten version of part of a thesis accepted for the degree of Ph.D. at the University of Cambridge.

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THE LAST SURGE OF THE NAUTILOID CEPHALOPODS

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Received March 9, 1949

Introduction

Recently it has been my privilege to make a general survey of the Tertiary nautiloids of the Americas and to study some large collections of comparable specimens from eastern and west-central Africa. As a result of this work, certain generalizations in regard to the post-Cretaceous development of these animals have become apparent, and in the following paragraphs I hope to outline them. Also it seems apropos for me to preface my remarks on this subject with a brief summary of the present beliefs in regard to the evolution of the nautiloids during the Paleozoic and the Mesozoic.

The Earlier History of the Nautiloids

It now seems reasonably certain that the nautiloid cephalopods made their debut fairly late in the Cambrian. During the Early Ordovician they evolved rapidly, and before the close of that epoch some of them had developed conchs which are so tightly coiled that their volutions are distinctly impressed dorsally, though uncoiling during late ontogenetic stages seems to have been more or less the rule. All of these early forms had orthochoanitic [1] or holochoanitic siphuncles, but cyrtochoanitic types were educed in the following epoch, the Middle Ordovician. At about the same time, or a little later, a maximum size seems to have been attained, and during the Middle and possibly the Upper Ordovician some of the straight nautiloids were ten or fifteen feet or more in length and were the largest animals extant—these were holochoanites, a group which probably became extinct near the close of the period. Also, during the last half of the Ordovician, there arose the peculiar mixochoanites, with siphuncles which are in part orthochoanitic and in part cyrtochoanitic in structure.

The Silurian, and particularly the mid-portion of it, was perhaps the heyday of the nautiloids, insofar as both numbers and kinds are concerned. As might be expected, progress was made along many lines, but it is difficult to point to any major structural advance that was achieved during this period. Furthermore, the mixochoanites became extinct near the end of the Middle Silurian.

Relatively few forms are known from the Lower Devonian, but the group prospered in the Middle and to a lesser extent in the Upper Devonian. Fairly early in this period, some form gave rise to the ammonoids, which evolved and multiplied rapidly and soon began to displace the nautiloids to a considerable extent. The ensuing eon of competition between these two groups continued throughout the remainder of the Paleozoic and all of the Mesozoic, though only the orthochoanites survived the close of the earlier era. Although locally nautiloids are not rare in the Late Paleozoic and according to Kieslinger (1925, pp. 101, 102) they reached a secondary climax in the Upper Triassic, for the most part, as Waagen, Spath, and others have pointed out, they are "not very common in Mesozoic formations, excepting a few species."

Cenozoic Development

Near the end of the Cretaceous, the ammonoids, like many other kinds of animals, suddenly became extinct, and their disappearance gave the nautiloids a

[1] For a glossary of nautiloid terminology, see Miller, 1947, pp. 21–23.

new lease on life. The eruptive response of the group to this last opportunity is the subject that I wish to discuss in particular. That is, at the very beginning of the Tertiary, the nautiloids seem to have undergone a development which was unparalleled in magnitude during all of the Mesozoic, though it was not as great as that which took place during the Early Paleozoic. Several genera arose almost simultaneously and a variety of large forms occurs in abundance in the early Tertiary at such widely separated localities as our Gulf Coastal Plain, northwestern Peru, and the northwestern part of Portuguese West Africa—one form is so abundant along the coast near Lobitos, Peru, that its type locality "has been named Punta Nautilus by the Lobitos geologists and engineers" (Olsson, 1928, p. 142). During the Eocene, nautiloids began to wane, and by the close of that epoch, or shortly thereafter, all of the genera but two, *Aturia* and *Nautilus* s. s., had become extinct. In the Oligocene and the Miocene, representatives of *Aturia* are not particularly rare in many parts of the world. However, Pliocene and Pleistocene nautiloids are almost, if not entirely unknown, and today only a tiny remnant of this once great race is left, several species of *Nautilus,* which live in the South Pacific.

Although it does not appear to have been emphasized previously, it now seems likely that the nautiloids almost became extinct at the close of the Mesozoic, and only one genus, *Eutrephoceras,* is definitely known to cross into the Cenozoic. It is a more or less generalized type characterized by a globular conch and almost straight external sutures, and it is the only nautiloid genus that was abundant and widespread during the Upper Cretaceous. Shortly before or shortly after the beginning of the Tertiary, depending upon how we correlate certain beds, this genus gave rise to species in which the conch is a little narrower and the sutures form rather prominent saddles on each side of the umbilical seams (fig. 2B). These constitute *Ci-*

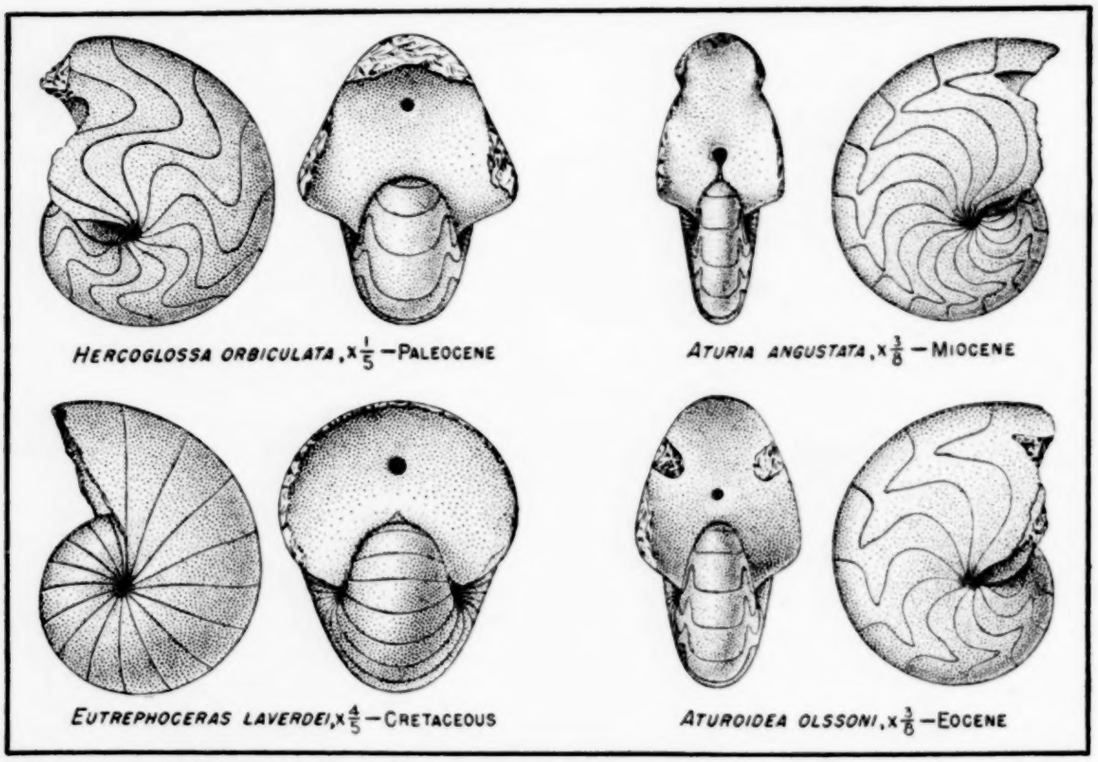
HERCOGLOSSA ORBICULATA, x 1/5 — PALEOCENE
ATURIA ANGUSTATA, x 1/8 — MIOCENE
EUTREPHOCERAS LAVERDEI, x 4/5 — CRETACEOUS
ATUROIDEA OLSSONI, x 1/6 — EOCENE

FIG. 1. Representative Cretaceous and Tertiary nautiloids (internal molds). Actual reduction 37 per cent greater than indicated.

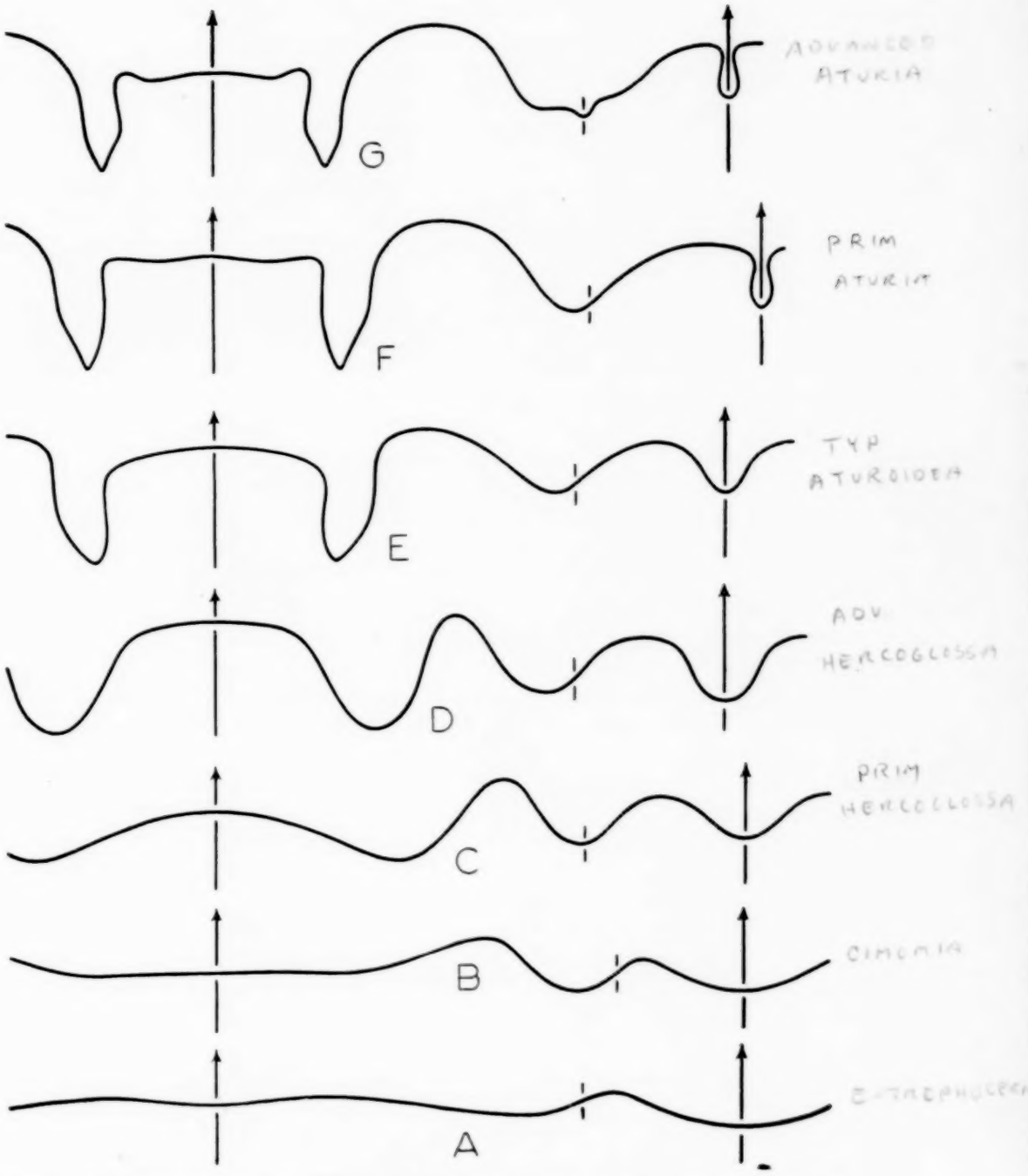

FIG. 2. Diagrammatic representations of mature sutures of *Eutrephoceras, Cimomia, Hercoglossa, Aturoidea,* and *Aturia.* **A**, a typical *Eutrephoceras, E.* aff. *E. dekayi* (Morton) from the Upper Cretaceous of South Dakota, × 3; **B**, a typical *Cimomia, C. vincenti* Miller from the Paleocene of Cabinda, × ⅜; **C**, a primitive *Hercoglossa, H. harrisi* Miller and Thompson from the Paleocene of Trinidad, × ¾; **D**, an advanced *Hercoglossa, H. orbiculata* (Tuomey), from the Paleocene of Alabama, × ¼; **E**, a typical *Aturoidea, A. paucifex* (Cope) from the Lower Eocene of New Jersey, × ¾; **F**, a primitive *Aturia, A. alabamensis* (Morton) from the Upper Eocene of Nuevo Leon, × 3; and **G**, an advanced *Aturia, A. curvilineata* Miller and Thompson, from the Miocene of Venezuela, × ⅜.

momia, a genus that is gradational with its presumed descendant, *Hercoglossa,* in which the conch is similar in shape, but the inflections of the sutures (particularly the ventral lobe) are greater. *Cimomia* most probably does not occur in strata older than Paleocene, and therefore I suspect that the Upper Jurassic specimens which have been referred to *Hercoglossa* are homeomorphs of typical representatives of that genus—in 1927 Spath (pp. 25–26) suggested that they "probably form an independent development."

The *Cimomia-Hercoglossa* stock gave rise to four quite diverse genera: *Deltoidonautilus, Woodringia, Aturoidea,* and *Nautilus.* The first of these differs from typical *Hercoglossa* only in that its conch is subangular rather than rounded ventrally and its volutions are therefore deltoid in cross section (impressed zone disregarded). Presumably this modification was moderately successful for the genus became essentially world-wide in its distribution. Nevertheless, it appears to be confined to the Paleocene and the Eocene, and it disappeared without leaving any recognizable descendants.

Woodringia also is close to *Hercoglossa* differing from it materially only in that its sutures form a slight median lobe in the ventral saddle. Both of the known representatives of this genus are from the Paleocene of North America. Presumably it, like *Deltoidonautilus,* was not in the main line of development of Tertiary nautiloids, and it does not seem to be particularly significant.

Except in longevity, by far the most successful progeny of *Hercoglossa* was the *Aturoidea-Aturia* stock. *Aturoidea* has a narrower conch than its ancestor, but the chief difference between these two genera lies in the sutures (cf. figs. 2D and 2E). That is, in *Aturoidea* the lateral lobes are relatively narrow and are subangular, and the lateral saddles of the external sutures are correspondingly wide. In *Aturia,* the end member of this series, the conch is still narrower; the camerae are relatively short; the sutures have attenuate lateral lobes, a medianly flattened ventral lobe, and a closed dorsal

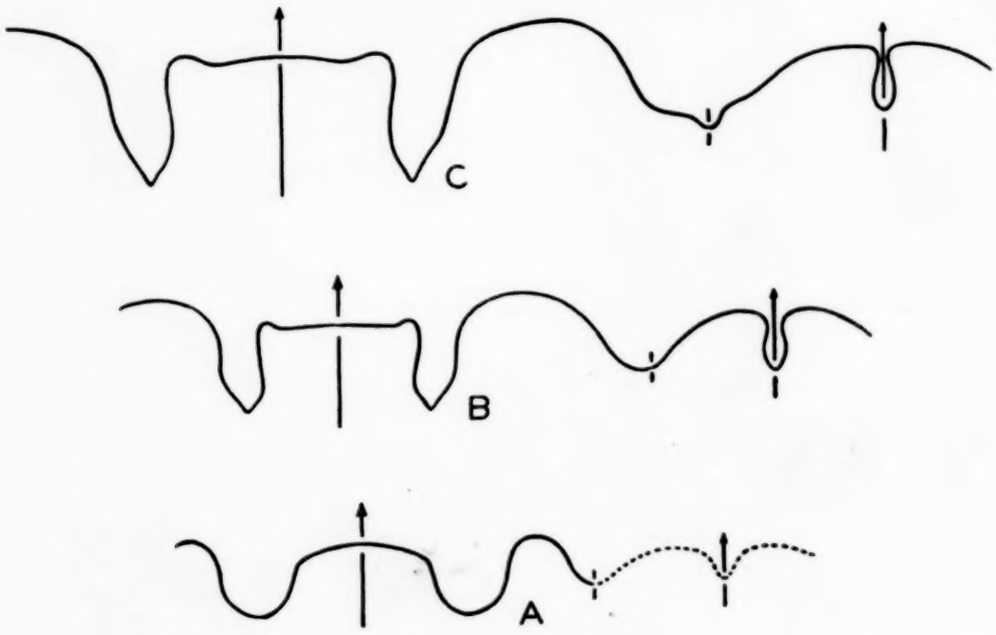

FIG. 3. Diagrammatic representations of early adolescent, late adolescent, and early mature sutures of *Aturia luculoensis* Miller, from the Eocene of Portuguese West Africa, × 15, × 3, and × 1½, respectively. **A** illustrates the first suture, **B** a suture where the conch is about 13.5 mm. in diameter, and **C** where it is about 40 mm. in diameter.

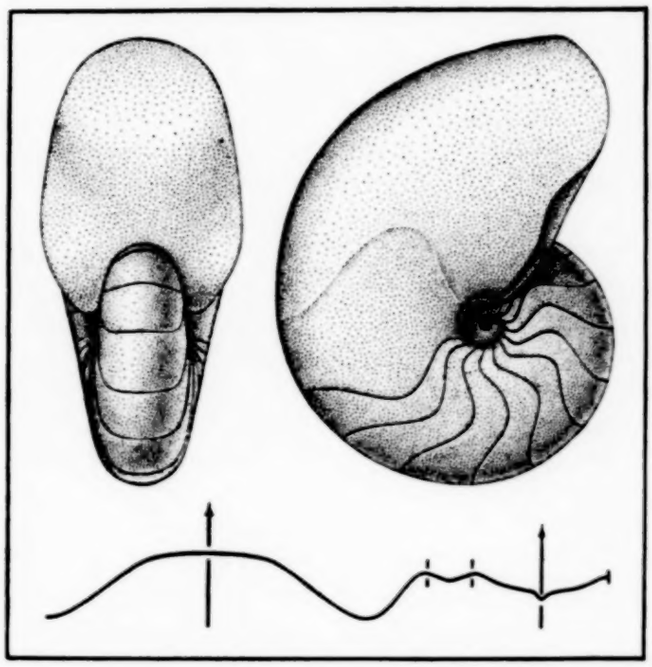

FIG. 4. An artificial internal mold of a typical modern representative of *Nautilus*, *N. pompilius* Linné, prepared by dissolving in hydrochloric acid a shell that had been filled with paraffin, × ⅓; and a diagrammatic representation of a mature suture of the same species, × ½.

lobe; and the siphuncle is subdorsal (that is, marginal) in position—in all of the other genera of Tertiary nautiloids the siphuncle tends to be subcentral. In advanced representatives of *Aturia*, the umbilical portions of the sutures are modified by the introduction near the umbilical seam of a small secondary lobe in the bottom of the umbilical lobe (fig. 2G)—this secondary lobe is the result of the dorsolateral portions of the septa becoming attenuate.

As is well known, in coiled cephalopods the inner volutions of the conch provide an excellent opportunity for us to check ontogeny against presumed phylogeny. Text figure 3 shows diagrammatically how the ontogenetic development of the sutures in *Aturia* confirm the belief that this genus arose from *Hercoglossa*, for the first suture in a typical species of it does not differ essentially from those of mature representatives of the latter genus. This figure also makes it clear that during late adolescence, advanced forms of *Aturia* have sutures like those of primitive congeneric species at full maturity.

Aturia is known to have been worldwide in its distribution, and representatives of it have been found on all of the continents and many of the oceanic islands. Stratigraphically it ranges from near the beginning of the Tertiary well up into the Miocene, after which nautiloids almost became extinct. In the Eocene of our Atlantic-Gulf Coastal Plain, where *Aturia* is abundant, it attains a large size, and phragmacones are known from there which have a diameter of as much as 35 cm.—the living chamber was at least half a volution in length, so the complete conch must have reached a diameter of more than 60 cm. In the post-Eocene strata of the same region, nautiloids are extremely rare, but the Oligocene and Miocene beds of our Pacific coastal region and those of Eurasia have yielded a good many representatives of *Aturia*. A few fairly

large individuals are known from the Oligocene of California.

It should be emphasized that the genus *Nautilus* is not an end member of this *Cimomia-Hercoglossa-Aturoidea-Aturia* series, but is a rather primitive form. It almost certainly developed from *Eutrephoceras,* possibly through *Cimomia.* No representatives of it are known from the Americas. In the eastern hemisphere, however, it has been found in beds that are as old as Eocene (Spath, 1927A, pp. 21, 23; 1927B, pp. 426–428). Nevertheless, throughout the Tertiary it was rare, no Pleistocene specimens have been reported, and at present it is almost extinct, being represented by only a very few closely similar species which inhabit the moderately shallow waters about the shores and coral reefs of the South Pacific from the Philippines and probably western Australia to the Fiji Islands. Empty shells drift to such remote places as Japan, Africa, and New South Wales.

The available data seem to indicate that, with the possible exception of *Nautilus,* all of the genera of nautiloids that are exclusively Tertiary appeared at about the same time. At least, all of them are known to occur in the Paleocene, though to be sure there is little unanimity of opinion as to the world-wide correlations of the strata which lie near the Mesozoic-Cenozoic boundary. The postulated phylogeny outlined above is therefore based almost entirely on shell morphology and not on chronology, but it should be emphasized that chronology does not militate against it and locally, at least, can be said to support it. For example, on our Atlantic-Gulf Coastal Plain, where the Tertiary strata have received a great deal of careful study, *Eutrephoceras, Cimomia,* and *Hercoglossa* are known from the oldest beds, the Midway group; and their presumed descendants, *Aturoidea* and *Aturia,* did not make their appearance in that region until the Eocene (Stenzel, 1940, p. 735). However, both of those genera seem to be represented in beds at least as old as the Midway in our Pacific Coast area and in the eastern hemisphere (see, for example, Schenck, 1931, pp. 438, 451–452, 453; and Spath, 1927A, p. 22).

Although it may be more apparent than real, there seems to be a certain parallelism between the development of the Tertiary nautiloids and the Upper Devonian ammonoids known as the clymenids, or better, the Intrasiphonata. Spath (1927A, p. 25) has called attention to the fact that in the Hercoglossidae "the suture-line has become 'goniatitic,' chiefly by the appearance of a distinct saddle on the inner lateral area." Furthermore, in *Aturia,* which is believed to have evolved from the Hercoglossidae, just as

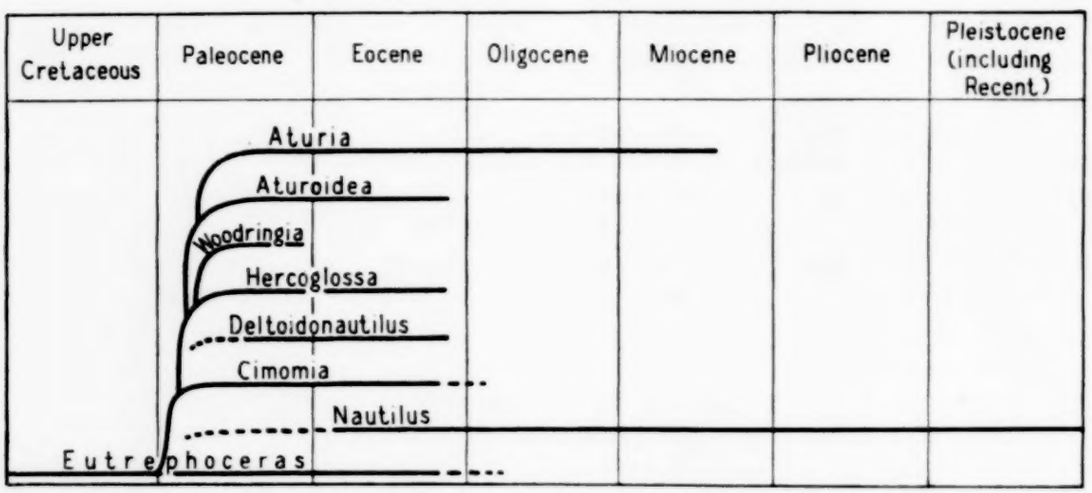

FIG. 5. Eruptive evolution of the Tertiary nautiloids. All of the genera (with the possible exception of *Woodringia*) appear to have been of world-wide distribution.

in the Intrasiphonata, the siphuncle became located close to the dorsum, and the sutures characteristically form prominent ventral saddles and a few pointed lobes separated by rounded saddles. These two groups are of course unrelated, but one can not help but wonder if the cause of the development was not the same in each case for it seems likely that both had similar habits and habitats.

Most probably the inflections of the septa in both nautiloids and ammonoids were developed in response to a need for a strong but at the same time a lightweight shell, and not as a device to enable the posterior portion of the animal to become more firmly attached to the shell. However, Newell (1949, p. 115) has recently suggested "that elaboration of the septa, reflecting crenulations of the posterior folds of the cephalopod mantle, may to some degree represent progressive modifications for increased efficiency in some physiological function such as, for example, respiration." Presumably strength was needed to allow the animals to go to moderately great depths, and possibly to protect them against the beating of the waves, and lightness (in weight) is of course invariably a desirable character for mobile creatures. As the inflections of the sutures (and therefore the septa) became progressively deeper, the shells of the Tertiary nautiloids became narrower almost certainly to facilitate motion through the water. This conclusion is substantiated by the fact that the most advanced of the Tertiary nautiloids, *Aturia,* has a relatively thin test, and primitive forms like *Eutrephoceras, Cimomia,* and *Hercoglossa* have comparatively thick and heavy shells. These subglobular forms must have been relatively poor swimmers. Modern *Nautilus,* in which the shell is intermediate in width, is believed to be quite mobile, and the surface of the test of living individuals is almost invariably smooth, clean, and free of encrusting organisms. I am uncertain as to why the siphuncle, though subcentral in most nautiloids, became marginal in *Aturia;* but in the ammonoids, which also are thought to have been agile creatures, the siphuncle was marginal, though in the great majority of them it was ventral rather than dorsal in position. Almost certainly its shifting changed the center of gravity, and in *Aturia* it had long funnel-shaped connecting rings and was therefore relatively heavy.

Gigantism in these nautiloids seems to have prevailed well before complete extinction. That is, the largest known Tertiary forms lived during the Eocene. In this connection it might be well to mention that the largest of the holochoanitic nautiloids lived during the Middle Ordovician and the largest of the cyrtochoanitic forms during the Lower Carboniferous—the holochoanites did not become extinct until the close of the Ordovician or shortly thereafter, and the cyrtochoanites continued to exist throughout most of the Permian.

The cause of the dwindling of the nautiloids, like that of the disappearance of the ammonoids, is unknown. The main line of development during the Tertiary culminated in *Aturia,* which quickly climaxed and then slowly dwindled to extinction. *Nautilus* is a relatively primitive form that is lingering long after the disappearance of its more advanced and probably in many ways more highly specialized relatives.

In concluding, I wish to acknowledge my indebtedness to Mr. Howard E. Webster of Iowa City for preparing the accompanying illustrations, and to the Graduate College of the State University of Iowa and particularly Mr. Frederick O. Thompson of Des Moines for making the completion of the manuscript financially possible. It should also be stated quite frankly that in the preparation of this study I have drawn freely from the monograph on the Tertiary nautiloids of the Americas, which the Geological Society of America recently did me the honor to publish; and the works of Drs.

A. A. Olsson, H. G. Schenck, L. F. Spath, and H. B. Stenzel have been most helpful.

Summary

Nautiloids appeared late in the Cambrian, evolved rapidly during the Ordovician, and climaxed in the Middle Silurian. Early in the Devonian they gave rise to the ammonoids, with which they had to compete during the remainder of the Paleozoic and all of the Mesozoic.

Near the close of the Cretaceous the ammonoids became extinct, and shortly thereafter the nautiloids underwent eruptive evolution. Only one genus, *Eutrephoceras,* is definitely known to cross from the Mesozoic into the Cenozoic. Early in the Tertiary it gave rise to *Cimomia* which is gradational with its presumed descendant, *Hercoglossa.* From this stock arose four quite diverse genera: *Deltoidonautilus, Woodringia, Nautilus,* and *Aturoidea.* The first two of these soon disappeared without leaving any recognizable descendants, and the third, though never abundant, continues to the present as a relatively primitive unspecialized form. The fourth, *Aturoidea,* almost immediately after its inception gave rise to *Aturia,* which is in many ways the most advanced of the Tertiary nautiloids. It achieved world-wide distribution and is known to have ranged throughout essentially all of the Tertiary but the very last part, the Pliocene.

The development of *Aturia,* which in certain ways is similar to the Intrasiphonata, is believed to have come about in response to a need for a strong but nevertheless light-weight shell. Gigantism, which in each of the chief groups of nautiloid cephalopods seems to have preceded extinction with a considerable margin, occurred during the Eocene. The cause of the dwindling of the nautiloids following their Paleocene surge is unknown.

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GENETIC ANALYSIS OF THE POLYMORPHISM OF COLOR PATTERN IN *DROSOPHILA POLYMORPHA*[1]

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Received April 11, 1949

Introduction

Natural populations of most species of Drosophila are very uniform in external morphology. This uniformity is a serious drawback of Drosophila as material for studies on population genetics. Since genetic analysis is predicated upon existence of genotypic variability among materials to be studied, traits other than externally visible structures had to be used in genetic population studies on Drosophila. Variations in the gene arrangement in chromosomes, and recessive genes carried in populations concealed in heterozygous condition, have been investigated by many authors. These investigations have led to great advances in our understanding of the evolutionary mechanisms that operate in Drosophila as well as of general problems of population genetics. The labor involved in detection and study of chromosomal variants and of concealed recessive genes is, however, considerable; a species of Drosophila with genetically determined discontinuous variability in external traits in natural populations would have a clear advantage over species hitherto used. Two such species are known, *Drosophila polymorpha* Dobzhansky and Pavan (1943) and *D. montium* de Meijere (see Duda 1924); both show heritable variations in the color pattern of the abdomen. In the first of these two species the variation is caused almost entirely by a single gene, the heterozygotes and the two homozygotes being phenotypically recognizable. The present article reports the results of a study of populations of *D. polymorpha* which inhabit the states of São Paulo, Rio de Janeiro, and Paraná in Brazil, and of artificial populations maintained under laboratory conditions.

Material and Methods

Samples of natural populations of *D. polymorpha* have been obtained by a method devised for collecting Drosophila under Brazilian conditions by my colleague, Dr. C. Pavan, and myself. Fermenting bananas are placed on the ground in protected locations, the space around this bait is cleared of twigs and branches, and the flies that are attracted are caught in an entomological collecting net. The material used in the work has been collected by Drs. C. Pavan, E. Nonato and Mrs. Martha Breuer as well as by the writer.

Unfortunately, *D. polymorpha* does not develop well on standard media for Drosophila culture. Although eggs are deposited abundantly, many larvae leave the food, wander on the glass of the culture vessel, and die before pupation. Attempts to remedy this defect by adding to the medium juices of several fruits and some vitamin concentrates have failed. Since the species develops slowly (about 30 days at 21°), infection of cultures by fungi, bacteria, and mites must be carefully avoided. Most experiments have been carried out at 21°–22°, in a cold room kindly supplied by the Rockefeller Foundation.

Color Types in *D. polymorpha* and Their Frequency in Natural Populations

Description of the pigmentation

Three types of color patterns on abdominal tergites can be distinguished in

[1] Contribution No. 2 of the cooperative research project of the University of São Paulo and the Columbia University on Genetics and Ecology of tropical Drosophila.

D. polymorpha, referred to as the dark, the light, and the intermediate types (Fig. 1, see also figures in da Cunha 1946). In the light type the posterior marginal bands on the abdominal tergites are very narrow and always interrupted in the middle. In the females, the band of the 1st tergite is absent, that of the 2nd extends laterally to about one third of the width of the tergites, on the 3rd to about the middle, on the 4th to about two thirds, the 5th has only a trace of a band, and the posterior ones no bands. In the males, the 1st tergite has no band, the 2nd only a trace, the 3rd and the 4th have narrow bands on the median half, the 5th has dark spots laterally from the middle, and the posterior tergites are light.

In the dark type the bands are much broader than in the light, their width being up to half of the length of the respective tergites in the middle part, the bands extending up to the whole width of the tergites laterally. The two last tergites are completely black in both sexes.

The color patterns are much less variable in the light and dark types than in the intermediate one. In the intermediate type the bands, especially on the 4th and 5th tergites, may or may not reach the anterior margin of the tergite laterally. If the bands do reach the an-

Fig. 1

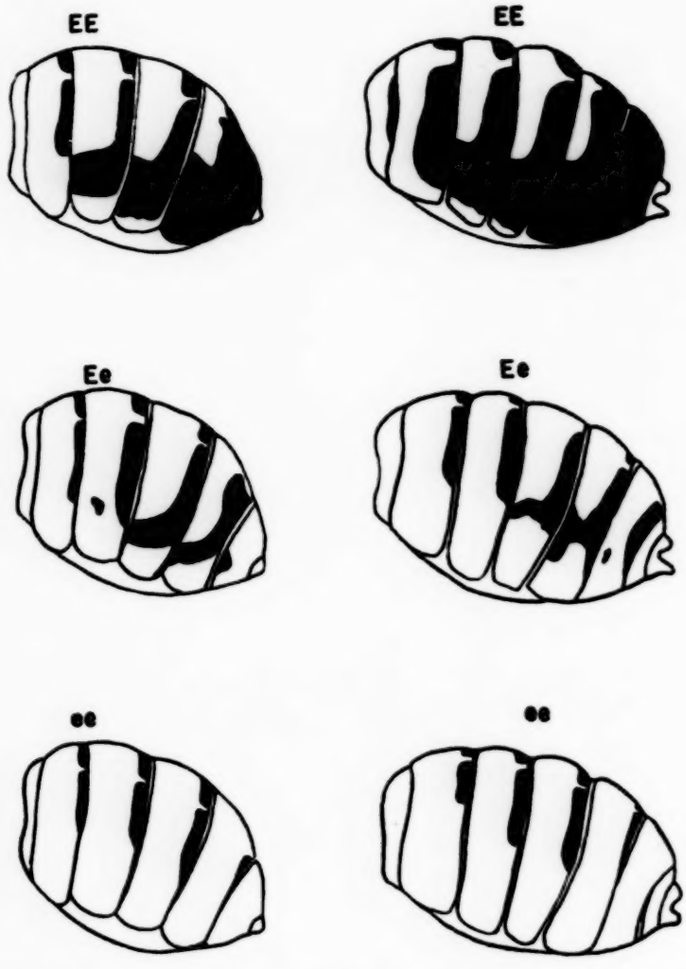

FIG. 1. Phenotypes of dark (EE), intermediate (Ee), and light (ee) color types in *Drosophila polymorpha.* Male left, female right.

terior margin they always have large yellow spots on the lateral margins, which fact distinguishes the intermediate from the dark type. The antero-lateral projections of the bands can become separated in the form of spots. The lightest variants of the intermediate type differ from the light type mainly in the presence of obvious black bands on the posterior segments of the abdomen, and also by a greater width and later extension of bands on all segments. Cases of asymmetry of banding are common in the intermediate but not in the other two types.

Flies of all types hatch from pupae with the dark parts of the abdominal pattern being faintly gray instead of black. The pigmentation develops gradually during several days, but in a few aberrant individuals the bands never become fully black. All experimental flies were classified on the 6th day after hatching or later. Other variations observed are replacement of the yellow by a reddish pigmentation of the light parts, which happens only in individuals of the light type, and replacement of the solid black bands by irregular black splotches.

Genetics of the pigmentation

Pure breeding strains of the dark and the light types have been established by mating together females and males of the respective types. The following 235 crosses using flies of different geographic origin have been made:

Araras	—60 crosses of dark	♀ × dark	♂ =	All dark offspring
Araras	—60 crosses of light	♀ × light	♂ =	All light offspring
Aguas de Prata	—20 crosses of dark	♀ × dark	♂ =	All dark offspring
Aguas de Prata	—20 crosses of light	♀ × light	♂ =	All light offspring
Itaiê	—20 crosses of dark	♀ × dark	♂ =	All dark offspring
Itaiê	—20 crosses of light	♀ × light	♂ =	All light offspring
Angra dos Reis	—20 crosses of dark	♀ × dark	♂ =	All dark offspring
Angra dos Reis	—15 crosses of light	♀ × light	♂ =	All light offspring

The offspring remained uniformly dark or uniformly light also in F_2 and F_3 generations of the crosses carried beyond F_1; some strains of Araras origin have been kept for about 50 generations showing only dark or only light flies respectively. In contrast to this, crossing flies of the intermediate type results in the production of dark, intermediate, and light flies in the offspring. Crossing dark flies with light gives only intermediate offspring, regardless of which form is used as a female and which as a male. As shown before (da Cunha 1946) these results are consistent with the view that the color pattern of the abdomen is controlled by one pair of alleles (E–e) without dominance. The dark (EE) and the light (ee) types are the homozygotes, and the intermediate (Ee) is the heterozygote.

Da Cunha (1946) found however that the segregation ratios obtained in F_2 deviate from the expected 1:2:1, in having an excess of heterozygotes, and advanced the hypothesis that the homozygotes are, under the conditions of the experiments, less viable that the heterozygotes. Further experiments have confirmed this hypothesis. Table 1 shows a summary of the F_2 data from all the dark x light crosses made so far.

To test the hypothesis further, some crosses have been made not under standard culture conditions, which involve some overpopulation of the cultures, but under what was believed to be optimal conditions with only a single female ovipositing for 3–4 days in each culture bottle. If the excess of heterozygotes and the deficiency of homozygotes are due to differential mortality between egg and adult stage, the ratios obtained under optimal conditions should agree more closely with the Mendelian expectation. The results are shown in table 2.

It can be seen from table 2 that the deviation of the observed from the expected figures are smaller when the larval

TABLE 1. *Summary of counts in F_2 generation of crosses of dark × light flies under standard conditions*

	Dark (EE)	Intermediate (Ee)	Light (ee)	Gene frequencies	
				E	e
Observed	1605	3767	1310	0.522	0.478
Expected	1670.5	3341	1670.5	0.500	0.500
Deviation	−65.5	+426	−359.5	+0.022	−0.022

$\chi^2 = 134.251$. $P < 0.01$.

TABLE 2. *Summary of counts in F_2 generation of crosses of dark × light flies under optimal culture conditions*

	Dark (EE)	Intermediate (Ee)	Light (ee)	Gene frequencies	
				E	e
Observed	2306	4697	2235	0.503	0.497
Expected	2309.5	4619	2309.5	0.500	0.500
Deviation	−3.5	+78	−74.5	+0.003	−0.003

$\chi^2 = 3.725$. $P = 0.10 - 0.20$.

competition is weakened or eliminated. Nevertheless, even these data indicate a slight excess of heterozygotes. The viabilities of the three types seem to be Ee > EE > ee.

Results of the test crosses intermediate × light and intermediate × dark are summarized in table 3. These results are consistent with the ones obtained in F_2 generations, although the Ee × ee cross gave no significant excess of the heterozygotes.

TABLE 3. *Summary of the test crosses intermediate × light and intermediate × dark*

	Ee ♂ × ee ♀		Ee ♂ × EE ♀	
	Intermediate (Ee)	Light (ee)	Dark (EE)	Intermediate (Ee)
Observed	1009	963	1422	1537
Expected	986	986	1479.5	1479.5
Deviation	+23	−23	−57.5	+57.5
	$\chi^2 = 1.07$. $P = 0.30$.		$\chi^2 = 4.468$. $P = 0.02 - 0.05$.	

Some experiments have been made in order to determine whether the relatively great variability of the color pattern in the heterozygotes, Ee, has or has not a genetic basis. The results are inconclusive, but on the whole favor the view that the high variability of the intermediate type is chiefly environmentally induced. The frequent asymmetries observed in flies of this type also favor this view.

Frequency of the color types in natural populations

Population samples have been obtained in several localities and some localities have been sampled repeatedly. The results are summarized in table 4.

It can be seen that in all samples from southern Brazil (i.e., in all samples included in table 4 except that from Belem, which is in the equatorial region) the dark type is the commonest, followed by the intermediate, while the light type is the least common. In the small sample from Belem the order is intermediate > dark > light. Since the color types

are caused by a single pair of alleles, E–e, the data can be expressed in terms of gene frequencies, as shown in the last two columns in table 4. Except for Belem, the frequency of E varies from 0.667 (Lambedor, January 1947) to 0.82 (Itanhaem, April 1947), with no clear geographic trends discernible. At Belem the frequency of E is 0.611.

Repeated sampling in the same locality discloses that the relative frequencies of the color types, and hence of the genes controlling them, may change from time to time in the same population. Thus, at Lambedor the frequencies of E were 0.67 and 0.68 in January and June of 1947 respectively, and 0.74 and 0.73 in September 1947 and February 1948.

TABLE 4. *Observed number of the dark, intermediate and light types in natural populations, and numbers expected according to the binomial square rule*

Locality	Date	Dark (EE)	Intermediate (Ee)	Light (ee)	Gene frequencies	
					E	e
Lambedor (Paraná)	Jan. 47—Obs.	2351	2200	617	0.667	0.333
	Exp.	2298.72	2295.62	572.61		
		$\chi^2 = 8.612$	$P < 0.01$			
Lambedor (Paraná)	Jun. 47—Obs.	167	151	39	0.679	0.321
	Exp.	164.57	155.65	36.77		
		$\chi^2 = 0.1838$	$P = 0.70$			
Lambedor (Paraná)	Sep. 47—Obs.	268	166	42	0.737	0.263
	Exp.	259.56	182.39	32.04		
		$\chi^2 = 3.723$	$P = 0.05$			
Lambedor (Paraná)	Feb. 48—Obs.	1183	657	229	0.730	0.269
	Exp.	1104.01	814.77	150.20		
		$\chi^2 = 77.541$	$P < 0.01$			
Itanhaem (São Paulo)	Dec. 46—Obs.	34	19	12	0.669	0.331
	Exp.	29.18	28.74	7.08		
		$\chi^2 = 7.514$	$P < 0.01$			
Itanhaem (São Paulo)	Apr. 47—Obs.	241	74	24	0.820	0.180
	Exp.	227.94	100.07	10.98		
		$\chi^2 = 22.96$	$P < 0.01$			
Itaie (São Paulo)	Mar. 46—Obs.	26	19	7	0.683	0.317
	Exp.	24.25	22.51	5.41		
		$\chi^2 = 1.147$	$P = 0.30$			
Mogi das Cruzes (São Paulo)	Dec. 47—Obs.	53	25	10	0.755	0.245
	Exp.	50.16	32.66	5.17		
		$\chi^2 = 6.46$	$P = 0.01$			
Mogi das Cruzes (São Paulo)	Feb. 48—Obs.	368	199	50	0.757	0.242
	Exp.	354.03	226.62	36.27		
		$\chi^2 = 9.115$	$P < 0.01$			
Pirassununga (São Paulo)	Dec. 48—Obs.	275	148	53	0.773	0.226
	Exp.	284.17	166.12	24.27		
		$\chi^2 = 37.277$	$P < 0.01$			
Pirassununga (São Paulo)	Feb. 49—Obs.	171	115	41	0.698	0.302
	Exp.	159.24	137.66	29.75		
		$\chi^2 = 8.470$	$P < 0.01$			
Icem (São Paulo)	Jun. 47—Obs.	120	61	16	0.764	0.236
	Exp.	114.94	71.07	10.95		
		$\chi^2 = 3.976$	$P = 0.05$			
Monjolinho (Goyaz)	Nov. 48—Obs.	41	21	7	0.746	0.254
	Exp.	38.3	26.08	4.41		
		$\chi^2 = 3$	$P = 0.10$			
Belem (Pará)	Jun. 48—Obs.	29	68	6	0.611	0.389
	Exp.	38.45	48.96	15.58		
		$\chi^2 = 15.625$	$P < 0.01$			

Whether the changes are or are not cyclic and connected with seasons, as has been observed in *Drosophila pseudoobscura* in the western United States (Dobzhansky 1943), is not clear from the data. A difference has also been observed between the Itanhaem samples of December 1946 and April of 1947, and in Pirassununga between the samples of December 1948 and February 1949.

Knowing the frequencies of the alleles E and e in the populations, one can calculate, using the binominal square rule (the Hardy-Weinberg formula), the frequencies of the dark, intermediate, and light phenotypes expected in population samples from the localities visited. Such calculations are, based on two assumptions, namely (1) that there is no differential mortality of the carriers of the two genotypes, and (2) that the different color types interbreed at random. The expected frequencies are given in table 4 for each sample. It can be seen that the heterozygotes are less frequent, and the homozygotes more frequent, than they are expected to be according to the the binomial square rule. The situation found in natural populations stands in sharp opposition to that observed in the crosses made in the laboratory, where the heterozygotes are more, and homozygotes less, common than they should be according to the Mendelian rules. The meaning of this will be considered in the Discussion.

Relative viability of the color types in laboratory experiments

The data in tables 1–3 permit the calculation of relative viabilities of the heterozygotes and of the two homozygotes in the environments that obtained in our experimental cultures. It is convenient to take the viability of the heterozygotes, Ee, to be equal to unity. If no differential mortality would take place among larvae of the three genotypes in the cultures, the numbers of the homozygotes EE and ee would be equal, within limits of sampling errors, to one half of the numbers of the heterozygotes in the F_2 crosses, while in the back crosses homo- and heterozygotes would appear with equal frequency. The survival rates of the homozygotes are, thus, obtained by dividing their observed numbers by half the observed numbers of the heterozygotes in the F_2 experiments and by the observed numbers of the heterozygotes in the back crosses.

From the data in table 1 (F_2 obtained under stringent conditions) we obtain the following figures:

EE	Ee	ee
0.852	1.00	0.695

If the same computations are made for the analogous experiments in which the environment was more favorable for the larvae (table 2), a different estimate is obtained, namely

EE	Ee	ee
0.981	1.00	0.951

The differential mortality is greater under unfavorable conditions, as expected. The back crosses give, however, some discordant estimates, namely (from table 3)

EE	Ee
0.925	1.00

Ee	ee
1.00	0.954

Some preliminary experiments were made with the aim to compare the physiological properties of the hetero- and homozygotes under laboratory conditions. Recently formed pupae were taken from crowded cultures of pure EE and ee types, and also from the cultures of F_1 crosses between them. These pupae

were placed in vials with filter paper and the numbers of the adult flies that hatched were counted. The results were as follows:

900 pupae EE gave 718 flies, or 79.7%,
900 pupae ee gave 706 flies, or 78.4%,
800 pupae Ee gave 622 flies, or 77.75%.

The proportions of the pupae hatching are not significantly different in the three cases. Other experiments, to be reported in detail in another publication, suggest that the time of development (from egg to adult stage) is shorter in heterozygotes than in the dark homozygotes, and in the latter shorter than in the light homozygotes.

Randomness of mating

It is very important to ascertain that natural populations of *Drosophila polymorpha* are panmictic as far as the coloration of the abdomen is concerned. Therefore, experiments utilizing the multiple choice technique were performed. Five mature (aged 1 week or more) males of a given type were placed in vials with five equally old females of each of the three color types. In 2 to 3 hours, all the females were separated from the males and were tested for production of offspring. A summary of the results is shown in table 5. No significant deviations from randomness of mating are apparent.

TABLE 5. *Percentages of fertile females of the three types obtained in cultures in which these females were exposed to insemination by males of one of these types. Numbers of tested females are given in parentheses*

Males	Females			χ^2	P
	EE	Ee	ee		
EE	20% (100)	29% (100)	28% (100)	1.89	0.40
Ee	37% (60)	23% (60)	20% (60)	3.50	0.20
ee	32% (100)	21% (100)	34% (100)	3.37	0.20

NATURAL SELECTION IN EXPERIMENTAL POPULATIONS

Attempts were made to set up artificial populations of *D. polymorpha* in population cages of the type used by Dobzhansky (1947b) for *D. pseudoobscura*. These attempts were unsuccessful, because many larvae leave the food and die of starvation on the bottom of the cage. Accordingly, a different type of apparatus has been developed by Dr. C. Pavan and the writer for *D. polymorpha* (fig. 2).

This apparatus consists of six one-liter bottles, resting on a wooden support, and interconnected by means of a metallic chamber with six cylindrical branches to which the mouths of the bottles are joined by rubber tubes. Banana agar

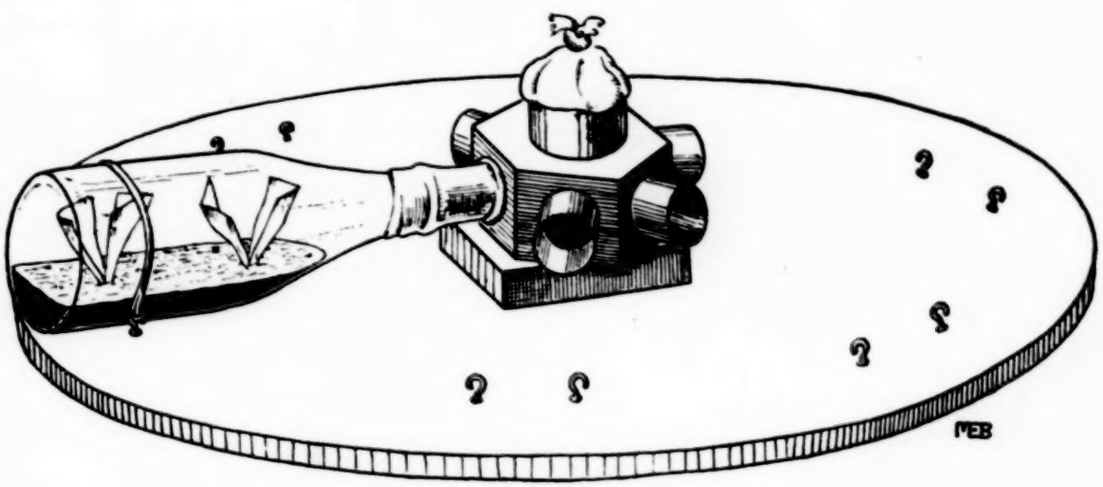

FIG. 2.

culture medium is poured into the bottles and permitted to solidify in horizontal position. Filter paper impregnated with a moldex solution is imbedded into the medium.

The experiments begin by placing a mixture of flies, with known proportions of the light and dark types, into a single bottle. To avoid inbreeding, the parental flies, numbering between 200 and 300, always come from several different dark and light strains. In about one week, a second bottle is added, and in another week a third. The experiments are carried at 21–22° C, at which temperature a generation of flies takes about 30 days. By the time the F_1 generation begins to hatch in the third bottle, the parents are almost all dead. A few days thereafter, the F_1 flies are etherized, classified, mixed, and returned back to the bottles. Depending upon the number of the F_1 flies hatched, one or two fresh bottles (the fourth and the fifth) are added, and the flies are made to pass into them by using their phototropism. One or two fresh bottles (the fifth and sixth) are added a week later; in a week more the oldest bottle is removed and a fresh one substituted. When the next generation (F_2) hatches, the flies are classified and counted, returned to bottles, and fresh bottles in which the F_3 is to develop are put on. Since the flies hatching in the oldest bottle are transferred to new ones only when at least two other bottles are producing adults, the generations are practically discrete and not overlapping. In this respect, our experimental design deviates from

TABLE 6. *Experimental populations starting with 20% dark individuals and 80% light individuals*

Generation	No. of exp.	No. of flies counted	Phenotype frequencies			Gene frequencies	
			EE	Ee	ee	E	e
F_1	9	2959	412	1613	934	0.411	0.589
			freq. obs. 0.139	0.545	0.315		
			freq. exp. 0.168	0.484	0.346		
			$\chi^2 = 42.79$				
F_2	7	2994	693	1589	712	0.496	0.504
			freq. obs. 0.231	0.530	0.237		
			freq. exp. 0.246	0.500	0.254		
			$\chi^2 = 11.255$				
F_3	9	4420	1198	2352	870	0.537	0.463
			freq. obs. 0.271	0.532	0.196		
			freq. exp. 0.288	0.498	0.214		
			$\chi^2 = 16.725$				
F_4	7	2853	934	1427	492	0.577	0.423
			freq. obs. 0.327	0.500	0.172		
			freq. exp. 0.332	0.489	0.179		
			$\chi^2 = 1.100$				
F_5	3	811	296	393	122	0.607	0.393
			freq. obs. 0.364	0.484	0.150		
			freq. exp. 0.368	0.477	0.154		
			$\chi^2 = 0.176$				
F_6	1	417	149	220	48	0.621	0.379
			freq. obs. 0.357	0.527	0.115		
			freq. exp. 0.385	0.470	0.143		
			$\chi^2 = 6.038$				

TABLE 7. *Experimental populations starting with equal numbers of dark and light individuals*

Generation	No. of exp.	No. of flies counted	Phenotype frequencies			Gene frequencies	
			EE	Ee	ee	E	e
F_1	18	4329	1467	2250	612	0.598	0.402
			freq. obs. 0.338	0.519	0.141		
			freq. exp. 0.357	0.481	0.162		
			$\chi^2 = 28.865$				
F_2	17	4369	1524	2170	671	0.597	0.403
			freq. obs. 0.348	0.496	0.153		
			freq. exp. 0.356	0.481	0.162		
			$\chi^2 = 2.546$				
F_3	16	6054	2161	2968	925	0.602	0.398
			freq. obs. 0.356	0.490	0.152		
			freq. exp. 0.362	0.479	0.158		
			$\chi^2 = 3.065$				
F_4	14	6244	2662	2745	837	0.646	0.354
			freq. obs. 0.426	0.439	0.134		
			freq. exp. 0.417	0.457	0.125		
			$\chi^2 = 9.681$				
F_5	10	4162	1792	1792	578	0.645	0.355
			freq. obs. 0.430	0.430	0.140		
			freq. exp. 0.416	0.457	0.126		
			$\chi^2 = 14.352$				
F_6	5	1814	851	655	308	0.649	0.351
			freq. obs. 0.409	0.361	0.169		
			freq. exp. 0.421	0.455	0.123		
			$\chi^2 = 77.438$				

that of Dobzhansky (l.c.) and approaches that of Reed and Reed (1948).

In eighteen experiments with the arrangement just described, the initial populations contained 50 per cent dark and 50 per cent light flies. These experiments were carried for from one to six generations, the proportions of the dark, intermediate, and light types being counted in each generation. In nine other experiments, the initial populations contained 20 per cent dark and 80 per cent light flies; in these experiments from one to six generations were obtained. Although the experiments were being made during about two years, some being started when others approached conclusion, the conditions were reasonably uniform, enabling us to treat the separate experiments as replicated trials, and to present their combined results in tables 6 and 7.

Inspection of the tables shows that in all experiments the proportions of dark flies rose at the expense of the light ones. The rates of change in the proportions of the dark and light types were relatively greater at the beginning of the experiments and decreased thereafter, the populations tending to reach equilibrium at about 40 to 45 per cent of the dark, about 35 to 45 per cent of the intermediate, and about 15 per cent of the light type. The gametic frequencies, q and $(1\text{-}q)$, of the genes E and e are shown in tables 6 and 7 in the two columns at the extreme right. In the populations in which the initial q is 0.2, it becomes doubled (0.41) in one generation, and reaches the value of 0.5 in two

or three generations. In populations with the original value of $q = 0.5$, the frequency of $q = 0.6$ is reached in one to three generations, and the equilibrium value, which is about $q = 0.65$, is practically attained in some four to six generations.

These results indicate that the intermediate type (the Ee heterozygotes) has the highest adaptive value, the dark type, EE, is next, and the light type, ee, is lowest in adaptive value. Taking the adaptive values of the three types to be respectively

$$(1-s)\text{EE} : 1\ \text{Ee} : (1-t)\text{ee},$$

the equilibrium frequency of E will be $\hat{q} = \frac{t}{s+t}$. By trial and error, it has been found that our experimental results are consistent with the following values of the selective coefficients: $s = 0.44$ and $t = 0.77$. A comparison of the observed and expected values of q is as follows:

Generation	Observed	Expected	Observed	Expected
Initial	0.20	—	0.50	—
1	0.41	0.37	0.60	0.56
2	0.50	0.49	0.60	0.59
3	0.54	0.55	0.60	0.61
4	0.58	0.59	0.65	0.62
5	0.61	0.61	0.65	0.63
Limit	—	0.64	—	0.64

It follows that, under the conditions of our experiments, the light type is effectively semilethal and the dark type considerably less well adapted than the heterozygote. This being the case, it is interesting to see whether the binomial square rule is realized among the flies obtained in the experimental bottles in various generations. If the great differences in the adaptive values of the three color types deduced from the changes observed in the experimental populations are due to differential mortality between the egg stage and the stage when counts of the adult flies are made, one can expect the heterozygotes (the intermediate type) to be more, and the homozygotes (light and dark) to be less frequent than demanded by the binomial square rule. This has been observed in the experiments of Dobzhansky on the behavior of chromosomal types in *D. pseudoobscura* in population cages. His experiments are in many ways analogous to ours. Tables 6 and 7 show the observed percentages of the three color types in each generation of the experiments, as well as the percentages expected on the basis of the binomial square rule with the gametic frequencies of the alleles E and e. The chi-squares, having one degree of freedom (Dobzhansky and Levene 1948), are also included in these tables.

The results are paradoxical. In the experiments beginning with the light type being in the majority ($q = 0.2$, table 6) significant excesses of the heterozygotes, and deficiencies of the homozygotes, are observed in the first three generations; in the fourth and fifth generations the observed and the expected values are equal within the limits of experimental error, and in the sixth generation a significant excess of heterozygotes is again observed. In the experiments beginning with equal proportions of the dark and light types (table 7) an excess of the heterozygotes is found only in the first generation, and excesses of homozygotes in the fourth, fifth, and sixth generations.

Discussion

Observations and experiments presented in this article show that the color variation in *Drosophila polymorpha* represents a case of balanced polymorphism. The three color types found in the species are produced by a single pair of alleles, the dark and the light being the homozygotes EE and ee respectively, and the intermediate the heterozygotes, Ee. The heterozygotes possess the highest adaptive value, followed by the dark homozygotes, while the light homozygotes are lowest in adaptive value. In other words

the adaptive values of the three types are: Ee > EE > ee.

However, since the progeny of two heterozygotes invariably includes some homozygotes, a natural population of a sexual species can consist exclusively of heterozygous individuals (no matter how high their adaptive value is) only if both homozygotes are lethal. If the heterozygote is superior and neither homozygote is lethal, natural selection will not be able to eliminate from the population the inferior of the two alleles. If the adaptive values of the homozygotes are not equal (EE > ee), the superior allele (E) will be more frequent than the inferior one (e). If the frequency of E is higher than 0.67, the most frequent type among the zygotes formed in populations at equilibrium will be EE (dark), followed by Ee (intermediate), while ee (light) will be the least frequent. Indeed, in natural populations of Brazil, the relative frequencies of the three types are dark > intermediate > light. In the experimental populations, regardless of whether the initial composition is 50 per cent dark: 50 per cent light, or 20 per cent dark: 80 per cent light, the frequency of the dark type increases until the equilibrium is reached at values corresponding to the gene frequencies $q = 0.64$ for E and $(1-q) = 0.36$ for e. The observations presented above attest, therefore, the effectiveness of the process of natural selection.

Attempts to clarify the mechanism of action of the selective process at work have not met with success. The simplest working hypothesis would be that the differences in the adaptive values of the three genotypes result in a differential mortality between the egg stage (or, more precisely, the formation of zygotes at fertilization) and the adult stage. If the carriers of the three genotypes mate at random, the zygotes will be formed, regardless of the distribution of the genotypes among the parents, in a ratio q^2EE : $2q(1-q)$Ee : $(1-q)^2$ee. However, if the survival rates of the three types are as $(1-s)$: 1 : $(1-t)$, this array will be transformed among the adults into $(1-s)q^2$EE : $2q\,(1-q)$Ee : $(1-t)\,(1-q)^2$ee.

With s and t positive, this means that an excess of heterozygotes and a deficiency of homozygotes should be found among the adult flies. This is precisely what is observed in the F_2 generation of crosses made in the laboratory, and also in some of the experimental populations, chiefly in those having low values of q. This situation is, consequently, analogous to that obtained by Dobzhansky in experimental populations of *D. pseudoobscura,* which contained mixtures of certain chromosome types. In accordance with this, Dobzhansky and Levene (1948) showed that excesses of heterozygotes and deficiencies of homozygotes are observed also in natural populations of *D. pseudoobscura.* Their findings in natural and in experimental populations are thus consistent. This is not the case in *D. polymorpha.* Paradoxically, natural populations of this species show excessive frequencies of homozygotes and deficiencies of heterozygotes. Deficiencies of heterozygotes appeared also in some of our experimental populations, chiefly in those in which the values of q approached the equilibrium frequencies.

The observed deficiencies of heterozygotes and excessive frequencies of homozygotes may, theoretically, be due to various causes. One may, for example, suppose that the different color types in natural populations do not interbreed at random, but that dark flies have a preference for mating with dark, and light with light, partners. Such preferential mating might result in frequencies of homozygotes higher than demanded by the binomial square rule, even though a differential mortality favors the heterozygotes. This hypothesis, though it gives a formally satisfactory account of the observations on natural populations, is contradicted by experiments which show that the color types interbreed at random. To resolve the apparent paradox, one

must suppose that the selective processes in populations of *D. polymorpha* involve not only a differential mortality of the three genotypes between the egg and the adult stage but also other differences between them. Indeed, mortality at any given stage of the life cycle is only one of the variables which determine the adaptive value of a genotype in a given environment. The adaptive value is the resultant of interaction of many variables, among which relative viabilities at different stages, the longevity of adult females and males, fecundity, and sexual activity are the most obvious ones. A genotype which suffers in competition with others at, for example, the larval stage, may nevertheless have a higher adaptive value because of, for example, a higher fecundity. This has been shown very clearly in the work of Wallace (1948) on the "sex-ratio" in *Drosophila pseudoobscura;* a genotype that has an advantage over another in one property may be equal or inferior in other aspects. A similar situation is suggested also for the gene arrangements in the third chromosome of this species by the results of Heuts (1948).

In the case of *D. polymorpha,* the differential mortality between the egg and the adult stage favors the heterozygotes in laboratory culture bottles and also, under same conditions, in experimental populations. But in natural populations, which evidently exist in environments different from those of laboratory cultures, differential mortality favors homozygotes. The agency which is responsible for this difference is unknown; a nutritional difference, involving different species of microorganisms, is a possibility. However that may be, the adaptive value of the heterozygotes is higher than that of the homozygotes both in natural and in experimental populations. Which physiological characteristics of the different stages of the life cycle of the fly are responsible for this remains unknown. Our results serve to emphasize the complexity of the property of the genotype referred to as its "adaptive value" which governs its evolutionary fate.

Summary

Three color types of the abdomen, a dark, a light, and an intermediate, exist in *Drosophila polymorpha.* Crossing experiments show that this variability is caused by a single pair of alleles, E–e, the dark (EE) and the light (ee) types being homozygotes, the intermediate heterozygous (Ee).

The ratios obtained in F_2 and in backcrosses made in laboratory bottles show deficiencies of homozygotes and excesses of heterozygotes, these deficiencies being greater under stringent and smaller under more favorable culture conditions. A differential mortality between the egg and the adult stage favoring heterozygotes evidently takes place.

Experimental populations have been maintained in a special apparatus described in the text. The initial populations contained either equal frequencies of the genes E and e, or else 80 per cent of e and 20 per cent of E. In either case, the frequency of E rose, until an equilibrium became established at about 64 per cent E and 36 per cent e. The rates of change are consistent with the assumption that, if the adaptive value of Ee is taken to be unity, that of EE is 0.56 and that of ee is 0.23.

Natural populations of various parts of Brazil always contain higher frequencies of the E than of the e allele. The frequencies vary however from place to place and from time to time, being evidently regulated by natural selection. Nevertheless, in natural populations the relative frequencies of heterozygotes are mostly below, and of homozygotes above, those demanded by the binomial square rule. Laboratory experiments indicate that the different color types interbreed at random. These observations suggest that, while in natural populations there occurs a differential mortality favoring the homozygotes, the heterozygotes nev-

ertheless have some advantages which give them a net superiority in adaptive value.

ACKNOWLEDGMENT

The author is deeply indebted to Professor Theodosius Dobzhansky for his help in the interpretation of the data and in the preparation of the manuscript. He is also grateful to Prof. A. Dreyfus and Dr. C. Pavan, and to Dr. Edmundo Ferraz Nonato, Mrs. Martha Breuer and Anita Nieckele and Mr. Henrique de Oliveira for their help during the work.

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CYTOLOGICAL EVIDENCE ON THE PHYLOGENY AND CLASSIFICATION OF THE DIPTERA

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Received March 11, 1949

INTRODUCTION

For several reasons the Two-winged Flies (Diptera) occupy a unique position in the field of evolutionary studies. In the first place this order of insects includes the genus *Drosophila,* whose genetic system has been investigated so intensively, in the early period (1914–30) from the standpoint of formal genetics and more recently from physiological and evolutionary viewpoints. *Drosophila,* however, is not the only genus of the Diptera upon which such studies have been carried out; several species of Fungus-gnats belonging to the genus *Sciara* have been investigated by Metz and his collaborators (general review in Metz, 1938), and preliminary work by Gilchrist and Haldane (1947) on *Culex* and Lerche (1941) and Bauer (1946) on *Phryne fenestralis* (see also Bauer and Lerche, 1943) suggests that other species of Diptera are highly suitable for genetic studies.

In the second place, it is only in the Diptera that one finds the giant "polytene" salivary gland chromosomes, which render possible a type of cytogenetic analysis that cannot be carried out in any other group of organisms. It is true that not all the Diptera possess chromosomes of this type, and that even among those that do the salivary gland chromosomes are seldom as favorable for a detailed analysis as they are in the genera *Drosophila, Sciara* and *Chironomus.* But even so, the fact that such an analysis can be carried out in many species of Diptera lends a special interest to all evolutionary studies on the group.

Lastly, as will be shown in detail below, the Diptera present several entirely different types of genetic systems whose relationship to one another constitutes in itself an evolutionary problem of considerable significance. With the possible exception of the Homoptera (Hughes-Schrader, 1948) no other insect order shows so many contrasting types of meiotic behavior, and the chromosome cycles of the Fungus-gnats (Sciaridae) and Gall-midges (Cecidomyidae) are entirely unique and exceed in complexity those of all other animals hitherto investigated by cytologists.

The classification of the Diptera has always presented a number of problems to systematists and no two authorities are agreed as to the relationships of the various groups. Attempts to classify the families into superfamilies or other higher categories have been mostly tentative and unsatisfactory. The purpose of the present paper is to discuss how far the cytological evidence can be made use of to establish the relationship of the various families within the order. The fact that the genetic system shows a highly unusual degree of variability within the order renders this possible; it would be impossible in most orders of insects, in which the genetic system has undergone fewer evolutionary changes.

ORIGIN OF THE DIPTERA

On morphological grounds the Diptera are currently regarded as belonging to the "Panorpoid Complex" of Tillyard, an assemblage which includes the orders Mecoptera, Neuroptera, Megaloptera, Raphidioidea, Zeugloptera (formerly included in the Lepidoptera), Trichoptera and Lepidoptera, together with a number of ill-defined Permian and Triassic groups such as the Paratrichoptera (Tillyard, 1919), Paramecoptera and Protomecoptera (Tillyard, 1918). It is pos-

sible that the Fleas (Aphaniptera or Siphonaptera) also belong to the Panorpoid complex, but their relationships are obscure.

It has been generally assumed in recent years that the Diptera arose (probably in Permian or Triassic times) from Mecopterous ancestors, the Triassic Mesopsychidae (Paratrichoptera of Tillyard) possibly representing an intermediate stage (Imms, 1931).

The cytological evidence on the origin of the Diptera, although far from conclusive, is interesting. On morphological grounds, the Crane-flies (superfamily Tipuloidea) are probably the most archaic type of living Diptera, so that it is their cytological characteristics that we have to take into account in considering the origin of the Diptera as a whole.

The genetic system of the Tipuloidea is characterized by the fact that meiosis in the male is "normal," chiasmata being formed in the autosomal bivalents. On the other hand the behavior of the X and Y chromosomes is somewhat unusual, since in *Tipula* (Bauer, 1931) and *Dictenidia* and *Phalacrocera* (Wolf, 1941) these elements do not pair to form a bivalent but arrange themselves on opposite sides of the equatorial plane at the first meiotic division so that they pass to opposite poles at the first anaphase. This mechanism, in which the sex chromosomes lack a pairing segment, but nevertheless disjoin regularly, is clearly a very specialized one. The only other order of insects in which it is definitely known to occur is the Neuroptera (Naville and de Beaumont, 1933, 1936; Oguma and Asana, 1932; Itoh, 1933; Kichijo, 1934; Klingstedt, 1937). This may suggest that the Diptera arose (presumably in Permian or Triassic times) from some group of Neuroptera or insects related to the Neuroptera, and that the peculiar sex chromosome mechanism which is still found in the living Tipuloidea was inherited from these "neuropterous ancestors." Such a view is in apparent conflict with the generally accepted theory of a Mecopterous origin for the Diptera.

The peculiar sex chromosome mechanism of the Neuroptera has been found in all the families investigated (Myrmeleontidae, Ascalaphidae, Osmylidae, Mantispidae, Chrysopidae, Hemerobiidae), with the single exception of the Sisyridae, in which a true XY bivalent is formed, according to Klingstedt (1936). The Snake Flies (Raphidioidea), generally regarded as a separate order related to the Neuroptera, show exactly the same type of "distance pairing" of the sex chromosomes, but some, at least, of the Megaloptera show an XY bivalent (Itoh, 1933).

It is unfortunate for the theory of the Mecopterous ancestry of the Diptera that so little cytological work has been carried out on the Mecoptera themselves. The species of *Panorpa* studied by Naville and de Beaumont (1934) were all XO in the males and nothing is known of the cytology of the other genera of Mecoptera (*Boreus, Bittacus, Merope, Chorista, Notiothauma,* et al.). If any XY Mecoptera exists it is quite possible that their sex-chromosomes show distance pairing, in which case this mechanism would characterize a large section of the more primitive members of the Panorpoid Complex—but there is no evidence on this point as yet.

We are inclined to agree with the opinion of Tillyard (1919) that the primary subdivision of the Diptera is into the Nematocera and Brachycera, rather than into the Orthorrhapha and Cyclorrhapha. In this classification, the Nematocera clearly represent the more primitive section of the Diptera, from which the Brachycera must have arisen at a later date. This is the position which has been taken by almost all recent authorities on the group, and there seems to be nothing in the cytological evidence to contradict it. We shall discuss later the question of which group of the Nematocera gave rise to the Brachycera.

Cytological Evidence on the Classification of the Nematocera

On cytological grounds the Nematocera can be divided into four groups (figs. 1 and 2). Each of these corresponds to a distinct type of genetic system. In spite of the fact that no genetical work has been carried out on any member of Group 1 and that the genetical work on *Culex* (Group 2) and *Phryne* (Group 3) is only preliminary, it is clear that the methods of inheritance and sex-determination differ considerably as between the four groups of the Nematocera.

Group I

Group I of our classification corresponds closely to the super-family Tipuloidea, recognized by most authorities on the group, except that, for a reason to be explained later, we exclude the Limoniidae from it. On morphological grounds the Tipuloidea seem to be a close-knit and entirely natural group, characterized by a V-shaped suture on the mesonotum, which does not occur in the other Nematocera. The cytological evidence suggests that the Tipuloidea are the most primitive of the Diptera, since they retain *both* the chiasmata in the male *and* the distance-pairing mechanism of the sex chromosomes—at least that is the case in *Dictenidia bimaculata* and *Phalacrocera replicata,* studied by Wolf (1941). In the Limoniidae on the other hand—or at any rate in *Dicranomyia trinotata* and *Thaumastoptera calceata* studied by Wolf (1941)—there are no cytologically distinguishable sex chromosomes, the chromosomal conditions resembling those found in the Culicidae, Chironomidae, Psychodidae and Simuliidae (Group II of our classification).

Tipula paludosa, studied by Bauer (1931), seems to resemble *Dictenidia* and *Phalacrocera* in its chromosome cycle, but some individuals possess 1–4 supernumerary chromosomes which seem to be at least partly homologous to the sex

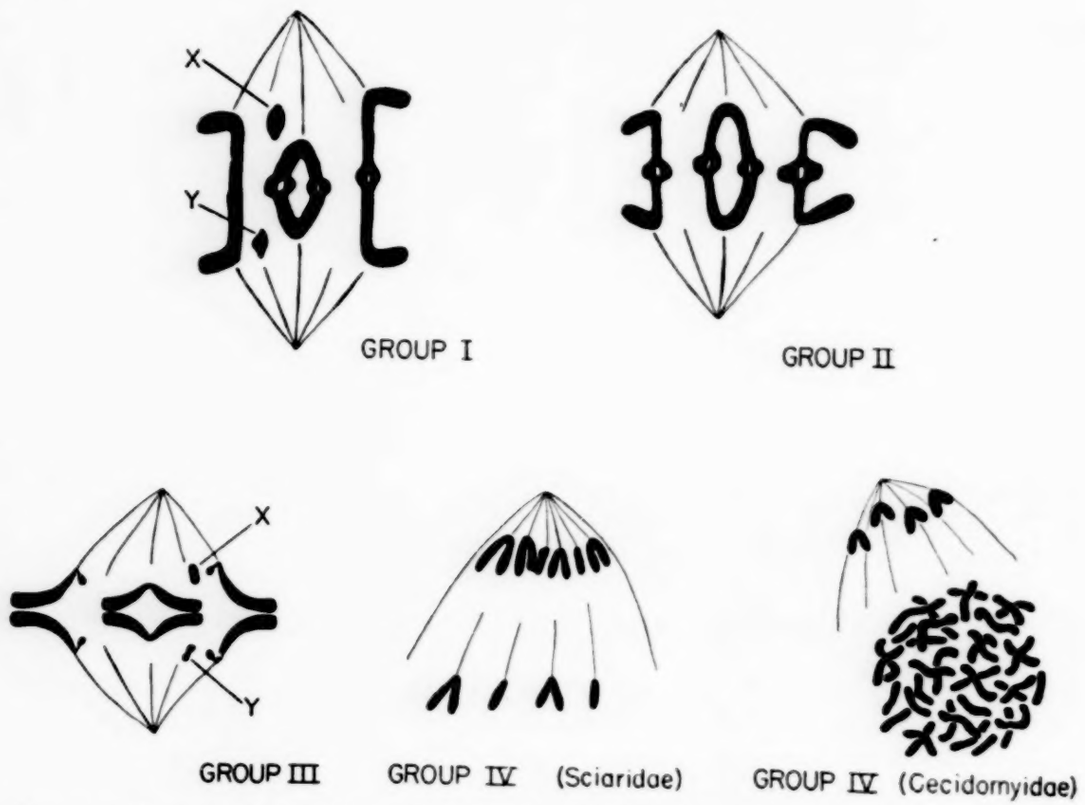

Fig. 1. Diagrams of the first meiotic division in the males of representatives of the four groups of Diptera Nematocera.

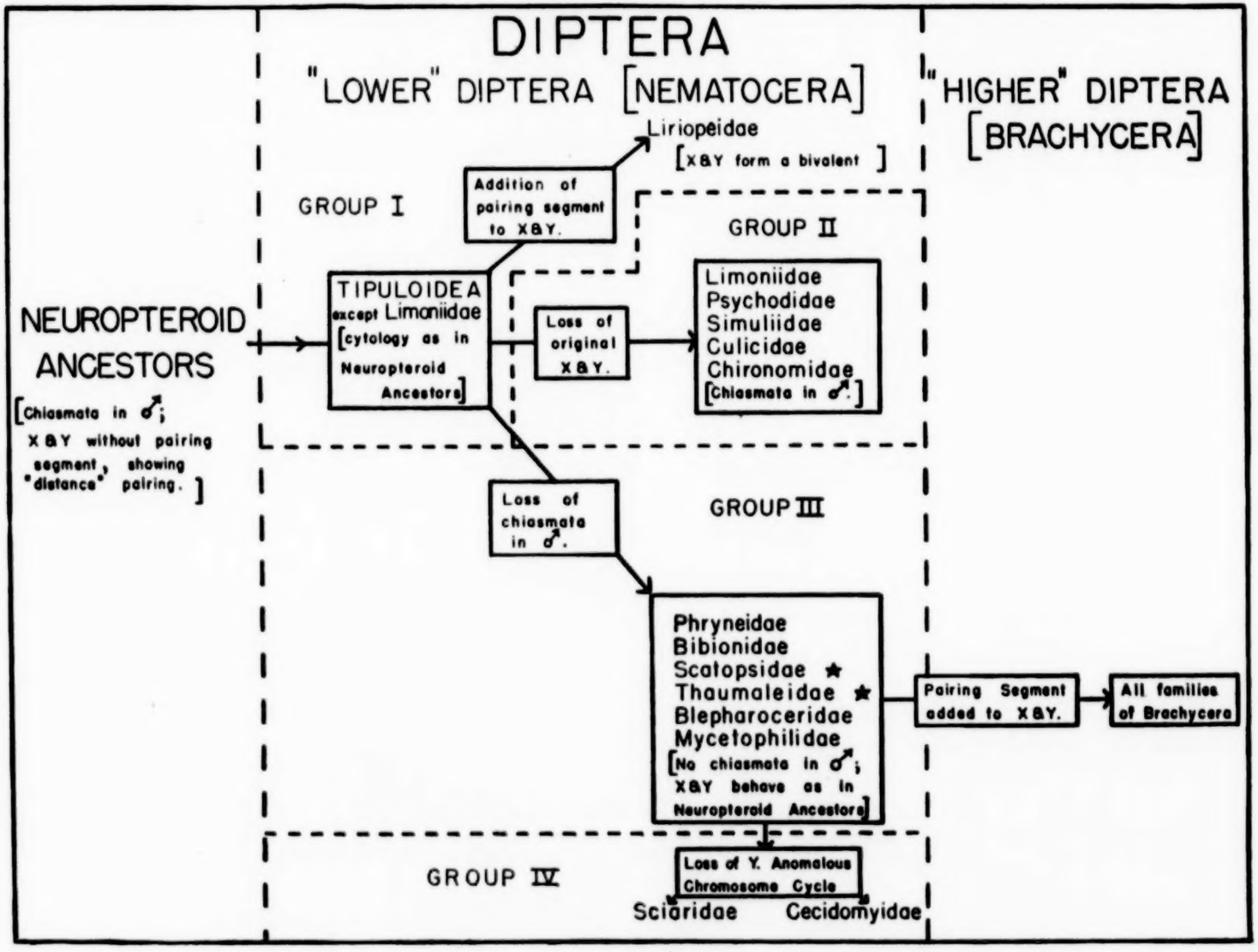

FIG. 2. Diagram of the classification of the Nematocera, based on the cytological evidence.
* Families in which the sex chromosomes are very minute. Further explanation in text.

chromosomes. Individuals carrying these extra chromosomes have a somewhat irregular meiosis in the male and may be partially sterile.

By some authors (e.g. Imms, 1926) the Tipuloidea are regarded as constituting a single family Tipulidae, but most modern authors (e.g. Curran, 1934) recognize four families (Tanyderidae, Ptychopteridae (= Liriopeidae), Trichoceridae and Tipulidae) or even six [the Cylindrotomidae and Limoniidae are usually looked upon as subfamilies of the Tipulidae, but have been regarded as distinct families by Brues and Melander (1932)]. With the exception of the Limoniidae, which the cytological evidence suggests are best regarded as a distinct family outside the limits of Group I, the Tipuloidea seem to be a cytologically homogeneous group, but Wolf (1946) has shown that in *Liriope* sp. (Ptychopteridae) the X and Y do not behave in the "neuropterous" manner, but pair to form a sex-bivalent at meiosis (although he was apparently unable to determine whether chiasmata were formed in this bivalent). It accordingly seems probable that somewhere in the ancestry of *Liriope* a "pairing segment" was added on to the original X and Y, probably through translocations involving a pair of autosomes. Changes of this type may be expected to occur from time to time in a group showing distance-pairing of the sex-chromosomes and are probably only of minor phylogenetic significance.

A more difficult question to answer is: what has happened to the X and Y in the Limoniidae? This question involves the origin of the families included in Group II from the Tipuloidea, or from some common ancestors having the same cytological characteristics as are shown by the majority of the Tipuloidea today. Clearly, the original X and Y were "lost" in the course of evolution, but how?

In order to answer this question, it is necessary to consider the evidence from the salivary gland chromosomes. Not only do the members of Group II lack any visible sex-chromosomes at meiosis —none of the authors who have studied the salivary gland chromosomes of the Chironomidae and Culicidae seem to have been able to detect any difference in banding which would indicate the presence of an X and Y. Either there is no "differential region" at all in the sex-chromosome pair, or this region is so short (comprising perhaps only one or two bands in the salivary chromosomes) that it has so far escaped detection. It is clear from the gynandromorphs which have been studied in a number of Culicidae and Chironomidae that a genetical sex-determining mechanism of some kind exists in these Diptera, and the genetical evidence of Gilchrist and Haldane indicates that it is the male which is the heterogametic sex, as in all those Diptera which have cytologically recognizable sex chromosomes. It seems probable that the transformation of the Group I sex chromosome mechanism into that found in Group II took place in two stages. First, the sex chromosomes became exceedingly minute, as they now are in the families Scatopsidae and Thaumaleidae (Group III). Then these minute elements were incorporated, by translocation, into one of the larger autosomal pairs thus losing their identity as separate chromosomes. It is at any rate easier to believe that such a transformation took place than to suppose that the Tipuloid sex chromosomes were actually "lost" and that an entirely new sex-determining mechanism took over. The transformation may have occurred in the Limoniidae (which on morphological grounds belong to the Tipuloidea, but on cytological grounds are members of Group II) or in some group allied to them.

Group II

This assemblage of families includes the Psychodidae (Montalenti, 1946), Simuliidae (Montalenti, 1947), Culicidae and Chironomidae. The Limoniidae also "key out" to Group II, but are morphologically members of the Tipuloidea. It is satisfactory to be able to place the

Psychodidae in a phylogenetic scheme, since this family has been regarded by Hendel (1928), Crampton (1925) and some other authors as a rather isolated one, of doubtful affinities.

The cytology of the members of Group II is remarkably uniform, nearly all of them having three pairs of metacentric chromosomes (a few Chironomidae and *Psychoda* sp. have four pairs). The Dixidae and Ceratopogonidae probably also belong to Group II, since all authorities are agreed in regarding the former as closely allied to the Culicidae and the latter as related to the Chironomidae—but nothing is known about the cytology of either family.

Group III

The families included in Group III are characterized by the fact that they do not form any chiasmata in the male, the bivalents being of the same type as those of Drosophila and the other Brachycera. The X and Y show the same type of "distance pairing" as in Group I, but in the Scatopsidae and Thaumaleidae both sex-chromosomes are exceedingly minute. The Bibionidae have been grouped with the Scatopsidae by most systematists, but the sex-chromosomes of *Penthetria holosericea* are very much larger than those of *Scatopse* spp. (Wolf, 1941).

The position of the Blephariceridae within the Nematocera has been much debated. These midges, which have an almost world-wide distribution, are associated with mountain streams and waterfalls, in which their specialized larvae live, attached to rocks by means of a series of suckers on the ventral surface of the body. Wolf (1946) has shown that in one species of this family (*Liponeura cinerascens*) the male lacks chiasmata and the X and Y fail to form a bivalent and show a type of distance-pairing. This definitely places the family in Group III and excludes any close relationship with the families in Group II.

The Anisopodidae (= Rhyphidae = Phryneidae) are another family whose systematic position has been much discussed. They are included in the Tipuloidea, somewhat doubtfully, by Curran (1934) and some other authors, and have been placed near the Trichoceridae. They lack the V-shaped suture on the mesonotum and, according to the cytological work of Bauer (1946), have no chiasmata in the male. They accordingly belong to Group III of our classification. Most authors have considered them to be a very primitive group, and they may well be ancestral both to the other families in Group III and to the "higher Diptera" (i.e. they may be the surviving representatives of the stock in which loss of chiasmata in the male first took place). This is the view adopted by Crampton (1925).

As shown by Frolowa (1929) and Le Calvez (1947) the Mycetophilidae (Fungivoridae of some authors) lack chiasmata in the male and have a pair of sex chromosomes which do not form a bivalent during spermatogenesis. This is significant, because on morphological grounds the Mycetophilidae and Sciaridae are obviously closely allied—so much so that they have been included in the same family by many European authors until recently. The fact that the Sciaridae have an exceedingly complex and anomalous type of chromosome cycle indicates that they are more widely separated from the Mycetophilidae phylogenetically than is apparent on the basis of their external morphology, and that those American entomologists (Curran, 1934, Shaw, 1948, etc.) who have regarded them as constituting a distinct family were entirely justified. It must be remembered, however, that we know nothing of the cytology of those Sciaridae which are not members of the genus *Sciara* itself—a cytological investigation of such genera as *Cratyna, Zygoneura* and *Epidapus* would no doubt yield interesting results.

Group IV

The fourth cytological subsection of the Nematocera comprises only the two families Sciaridae and Cecidomyidae. On grounds of external morphology, most

entomologists have grouped these two families together with the Mycetophilidae. A relationship undoubtedly exists but, as we have seen above, the Mycetophilidae have a relatively "orthodox" chromosome cycle, while in the Sciaridae and Cecidomyidae the genetic system has acquired the most bizarre complications. The chromosome cycle of *Sciara* has been reviewed by Metz (1938) and those of several Cecidomyidae by the author (White, 1946, 1947 *a*, *b*), so that no attempt will be made to discuss them in detail here. Briefly, the main features are these:

(1) In both families the sex of the individual depends on the elimination of certain X-chromosomes from the somatic nuclei (but not from those of the germ line) during the cleavage divisions.

(2) In both groups there are certain chromosomes which are present in the germ-line but not in the soma (these are few in number in *Sciara* and even absent altogether in some species, while in the Cecidomyids they are always more numerous than the chromosomes which are present in the soma).

(3) The first meiotic spindle in the male is a "unipolar" one in both groups.

This is an impressive list of resemblances, but the differences between the chromosome cycles of the two families are also striking. Thus, the female meiosis in *Sciara* is of an "orthodox" type, while in the Cecidomyidae it is highly peculiar (White, unpublished). Also, the meiosis of the male Sciaridae and Cecidomyidae is not of the same type, although in both there is no pairing of chromosomes and a unipolar spindle is formed at the first division.

Within the Cecidomyidae, the Lestremiinae are undoubtedly the most primitive subfamily. Unfortunately, nothing is known of their cytology as yet; probably their chromosome cycle is of the same type as that of the subfamilies Heteropezinae and Cecidomyinae but this requires confirmation.

Comparison with other classifications

It will be seen that our arrangement of the nematocerous families (fig. 2) agrees fairly well with that of Edwards (1925), which was based, of course, solely on external morphology. There are, however, some significant differences. The three subsections of the Nematocera recognized by Edwards (which he considered had been separate since the Jurassic, if not earlier) are as follows:

A

Mycetophilidae
Bibionidae
Scatopsidae
Cecidomyidae and their allies

B

Ptychopteridae
Psychodidae
Culicidae
Chironomidae, etc.

C

Trichoceridae
Tipulidae

Edwards clearly considered the Dixidae as members of his Group B (they are probably related to the Culicidae), but he was uncertain as to the position of the Blephariceridae, Deuterophlebiidae, Simuliidae and Thaumaleidae. The Anisopodidae (Rhyphidae, Phryneidae) he regarded as possibly near to the ancestral stock of all three groups.

It would seem that Edwards' Group A corresponds to our Groups III and IV, his Group B to our Group II (with the exception of the Ptychopteridae (Liriopeidae), which we are inclined to consider as aberrant members of Group I) and his Group C to our Group I. The families which Edwards regarded as of doubtful affinities can now (with the exception of the Deuterophlebiidae, which may or may not be closely related to the Blepharoceridae) be definitely assigned to their proper places, namely the Anisopodidae, Thaumaleidae and Blephariceridae to Group III and the Simuliidae to

Group II. Edwards believed that the "Brachycera and Cyclorrhapha" (Brachycera in our sense) arose from a primitive member of Group B which was related to the Anisopodidae. We consider, on the other hand, that the lack of chiasmata in the male clearly indicates that the "higher Diptera" originated from Group III of our classification, but since the Anisopodidae now appear to belong to this group (rather than to Group II) our conclusion is not so very different from Edwards' one.

The possibility that loss of chiasmata in the male originated on several distinct occasions in the phylogeny of the Diptera (i.e. that the character is a polyphyletic one) seems to us an extremely remote one. Certainly, one should not make such an assumption unless other evidence seemed to require it, which does not seem to us to be the case. Such a loss involved the replacement of one genetic system by a completely new one—a fundamental change of an entirely different nature to the "loss" of a single morphological character.

The Brachycera

The suborder Brachycera includes 96 families, according to Brues and Melander (1932), whereas the Nematocera only comprise about 30 families. Although the literature on the cytology of the Brachycera is fairly extensive it deals in the main with only four families (Asilidae, Calliphoridae, Drosophilidae, Hippoboscidae). The evidence, so far as it goes, tends to show that all the Brachycera lack chiasmata in the males. This is true of the Asilidae, a relatively primitive family of the group Orthorrhapha (Metz and Nonidez, 1921, 1923), as well as of the specialized Muscoid and Hippoboscid flies (Cooper, 1941, 1944). It thus appears probable that the Brachycera arose from that section of the Nematocera which had already lost the chiasmata from spermatogenesis; since the meiosis of the Sciaridae and Cecidomyidae is far too specialized for them to have given rise to the Brachycera, the cytological evidence indicates that the latter arose from our Group III of the Nematocera, a conclusion which is also reasonable on grounds of external morphology.

Except in the case of the Drosophilidae and the Hippoboscidae, studied by Cooper, the sex chromosomes of the Brachycera have not been studied in detail. It is clear, however, that apart from a few aberrant XO species (the Asilid *Dasyllis grossa* and a few species of Drosophila) all the Brachycera are XY in the males. Unlike Groups I and III of the Nematocera, however, the X's and Y's of the Brachycera seem to possess pairing segments, so that they form a true bivalent at the first meiotic division, in spite of the fact that no chiasmata are present. The pairing segment seems to be usually short, relative to the total length of the X and Y, i.e. both these chromosomes usually contain extensive differential segments. The pairing segments seem, in the majority of cases at any rate, to include the centromeres and are probably always composed of heterochromatic material. Darlington (1934) claimed that a reciprocal pair of chiasmata were regularly formed in the pairing segment of the XY bivalent of *Drosophila pseudoobscura*, but his claim has been disputed by Cooper (1944) and it now appears almost certain that no chiasmata are present in spermatogenesis, either in the autosomal bivalents or in the XY pair.[1] The only Brachyceran

[1] Cooper (1949) has described certain inconstant appearances in the autosomal bivalents of the male Drosophila which he refers to as chiasmata. Since, however, the genetical evidence indicates that these are not due to crossing-over (a fact recognized by Cooper), they are not chiasmata in the generally recognized sense of the term. Whatever their cytological interest, they do not affect the validity of the general statement that the males of all Brachycera hitherto studied appear, on genetical or cytological evidence (or both), to lack crossing over as a normal part of their genetic mechanism. The chiasmata of the Nematocera included in our Groups I and II are entirely typical and regular in their occurrence so that there is every reason to believe they are due to crossing-over, in spite of Cooper's doubts on this point.

(apart from the few XO forms mentioned above) which lacks pairing segments in its sex-chromosomes is the aberrant parasitic fly *Melophagus ovinus*, in which the X and Y show a type of distance-pairing at meiosis (Cooper, 1941); since this is a very specialized fly it is probable that it has lost the pairing segments found in the other Brachycera.

It seems, therefore, that in the evolution of the Brachycera the old "distance pairing" mechanism was lost from spermatogenesis, and that pairing segments were added to the X and Y, possibly by translocations of autosomal regions to the sex chromosome pair. The mechanism of spermatogenesis appears, on the basis of the evidence so far available, to be very uniform throughout the Brachycera, so that there is little hope of our being able to classify the families of higher Diptera on cytological grounds, in the same way as we have done for the Nematocerous families. But it is quite possible that a more detailed study of the sex chromosome pair in the families of Brachycera might reveal the existence of different types of X and Y chromosomes (differing in the extent and position of the pairing and differential segments) which could be made use of in classification.

Summary

The most primitive Diptera (Tipuloidea) are characterized by the presence of chiasmata (= occurrence of crossing over) in both sexes, and by the fact that the sex chromosomes do not form a bivalent in the meiosis of the male. In the latter feature they resemble the Neuroptera.

From this group two main evolutionary lines arose. One led, probably via the Limoniidae (or some group related to them), to the Psychodidae, Simuliidae, Culicidae and Chironomidae. In this group the chiasmata were retained in the male, but the sex chromosomes disappeared as distinct elements of the chromosome complex. In the other line the original sex chromosome mechanism was retained, but the chiasmata were lost from the male sex. This line led, probably via the Anisopodidae (Rhyphidae, Phryneidae), to such forms as the Bibionidae, Scatopsidae, Blephariceridae and Mycetophilidae. From this line there arose, probably at an early stage, the higher Diptera (Brachycera) in which a pairing segment has been added to the sex chromosomes. From the same line there also arose (probably from primitive Mycetophilidae) the aberrant Sciaridae and Cecidomyidae, in which the whole chromosome cycle has become highly anomalous.

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THE SELECTION INDEX AND ITS TEST OF SIGNIFICANCE

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Received March 4, 1949

Under experimental conditions in which equal numbers of individual animals of two types, A and B, are exposed to selection by a predator with the result that the numbers of individuals taken are a and b and the numbers not taken are c and d, respectively, making a combined total of n, then we may measure the strength of selection by the selection index: $(a - b)/(a + b)$. Should a greater number of type A than of type B individuals be taken (selection against A), then the selection index will be positive, while if more of B than of A are taken (selection against B), the index will be negative.

The formula which I earlier proposed (Dice, 1947:3) for the calculation of the chi-square of the difference between the numbers of A and of B taken under such conditions, unfortunately, is inappropriate. This has been pointed out to me by Don W. Hayne, who also has given other aid with this problem. The formula for chi-square earlier given, $(a - b)^2/(a + b)$, is correct for a 1:1 ratio, as stated, but that formula takes account only of the successes in each of the two classes and neglects the failures in the same classes.

The chi-square appropriate for testing the significance of the deviation index from zero is that for a 2×2 table:

$$\chi^2 = \frac{n(ad - bc)^2}{(a + b)(c + d)(a + c)(b + d)}.$$

Inasmuch, however, as under the conditions of experiment the numbers of individuals of each of the two types exposed to selection are equal, therefore, $a + c = b + d$, and accordingly:

$$\chi^2 = \frac{(a - b)^2}{(a + b)} \cdot \frac{n}{(n - a - b)}.$$

The chi-squares calculated according to the formula given in my earlier paper, therefore, need to be multiplied by $n/(n - a - b)$, with the result that in every case the significance of the selection observed will be greater than previously indicated. There is a single degree of freedom. This revised formula was suggested by C. W. Cotterman.

Another shortcoming in the statistical analysis presented in my earlier paper was the lumping of data obtained from experiments conducted concurrently on backgrounds of two contrasting colors. In these experiments the deer-mice (*Peromyscus maniculatus*) exposed to predation by owls were of two types of pelage color, one type matching more or less closely the color of the soil background in one compartment, the other type matching the soil color of the other compartment. Four mice of each of the two color types were exposed alternately in the two compartments. The degree to which the pelage colors of the presumably concealingly-colored prey animals matched their backgrounds, however, could not be made exactly the same in the two compartments. Furthermore, the pelage color of the one type of prey was usually more variable than that of the other type. In view of these sources of variation within the experiments it is desirable to measure the strength of selection separately for each color of background. No important additional information will be obtained by calculating an average selection index for the two differing colors of backgrounds.

The selection indexes and their chi-squares for the experiments on selection performed by Sumner (1934, 1935*a*, 1935*b*) and by Dice (1947) are presented in table I. For each experiment

the degree of selection obtained on each color of background is presented separately. The figures given refer in each case to the strength of selection against the type of animal which to the human eye appears to be conspicuous against the background used in the experiment. The experiments by Isely (1938) on the selection of acridians by various predators are not included in the table, because they do not lend themselves to simple statistical treatment by the methods here employed.

In calculating the selection indexes and chi-squares from the data obtained in those experiments by Sumner where a penguin was the predator, four individuals listed by him as of doubtful status have been omitted. In those experiments where a night heron was the predator, the animals injured by the predator have been included, along with those eaten, to form the total taken. From the series of experiments where the sunfish was the predator, those experiments have been omitted where the numbers of the two colors of prey offered were unequal and also those where a long waiting period ensued before exposure to the predator. This waiting period tended to allow the prey individuals to change their color.

In all the experiments performed by Sumner on the selection of mosquito-fish (*Gambusia patruelis*) whose colors were adapted to pale and to dark backgrounds, respectively, it will be noted that there was consistently a selection against the conspicuously colored animals. The selection indexes in the several experiments range from 0.22 to 0.57. The deviation from the 1 : 1 ratio expected in the absence of selection is highly significant in every experiment.

TABLE 1. *Summary of experiments on selection of prey on backgrounds of matching and of contrasting color*

Predator	Background	Total prey exposed	Number of prey taken		Selection index against conspicuous	$\frac{\chi^2}{1 \text{ d.f.}}$	Experimenter
			Concealingly colored	Conspicuous			
Prey: Mosquito-fish (Pale vs. dark)							
Penguin	Pale	572	103	165	.23	26.99	Sumner, 1934
Penguin	Black	470	73	201	.47	143.39	Sumner, 1934
Night heron	Pale	400	41	67	.24	8.57	Sumner, 1935a, Table 2
Night heron	Black	600	99	156	.22	22.16	Sumner, 1935a, Table 2
Sunfish	Gray	430	71	137	.32	40.56	Sumner, 1935b
Sunfish	Black	200	8	29	.57	14.62	Sumner, 1935b
Prey: Deer-mouse (Ivory vs. discard gray)							
Barn owl	Nearly white	960	32	58	.29	8.29	Dice, 1947, Table IV
Barn owl	Yellowish gray	960	36	66	.29	9.87	Dice, 1947, Table IV
Long-eared owl	Nearly white	528	17	14	−.09	.31	Dice, 1947, Table V
Long-eared owl	Yellowish gray	536	9	29	.53	11.33	Dice, 1947, Table V
Prey: Deer-mouse (Buff vs. *blandus* gray)							
Barn owl	Yellowish gray	352	25	55	.37	14.56	Dice, 1947, Table VI
Barn owl	Dark gray	352	40	52	.13	2.12	Dice, 1947, Table VI

The trend of selection is not so consistent in those experiments by Dice where owls were the predators and deer-mice of several color strains were the prey. In one of the experiments where the long-eared owl was the predator, more of the presumably concealingly colored individuals were taken than of the conspicuous ones, with a resulting selection index of $-.09$. The deviation from an index of 0.0, however, is not significant and may well have occurred by chance. In the reverse experiment with the same bird and the same colors of mice, but on yellowish gray soil instead of on nearly white soil, the high and very significant selection index of .53 was obtained. The long-eared owl was very shy and rather erratic in its behavior and this may account in part for the difference between these two selection indexes.

The barn owl was a much more consistent worker. This is shown by the more nearly uniform selection indexes, ranging from .13 to .37, in those experiments in which this owl served as the predator. The low and non-significant index of .13 obtained in one experiment was perhaps due in part to the failure of the gray mice to match very well the color of the dark gray soil and in part also to their considerable variability in shade of pelage color. In all the other experiments in which the barn owl was employed as the predator, selection against the conspicuously colored mice was highly significant.

In evaluating the selection indexes obtained in the last two experiments listed in the table (from Dice, 1947, table VI) consideration should be given to the fact that the contrast between the prey animals and their backgrounds was purposely made slight, so as to test the amount of selection which might occur between strains of mice which differ only slightly in pelage color. It is evident from the selection indexes obtained in these two experiments that selection may be operative in choosing between strains which exhibit variations in pelage color which are considerably less extreme than many which occur in nature.

In all the experiments listed in table I only two have selection indexes which are not significant. These are the ones with indexes of $-.09$ and of .13, respectively, which were previously mentioned. In the other ten experiments for which data are available the selection indexes range from .22 to .57 and all of these ten indexes are highly significant.

No modification need be made, therefore, in the conclusions previously reached (Dice, 1947), that, under properly controlled experimental conditions, predators tend in general to take a considerably greater proportion of those prey which appear conspicuous against their backgrounds than of prey which are concealingly colored. Should anything like such high rates of selection be effective in nature as are demonstrated in the laboratory, very rapid evolution in a variable population could result from the operation of natural selection.

Summary

When equal numbers of two types of prey, A and B, making a total number n, are exposed to predation, with the result that the number of individuals of each type taken are a and b, respectively, then the strength of selection against type A and in favor of type B is expressed by the selection index: $(a-b)/(a+b)$. The test of significance previously proposed (Dice, 1947) for the deviation of the selection index from zero, however, is erroneous. The appropriate formula is: $\chi^2 = [(a-b)^2/(a+b)][n/n-a-b]$. Revised calculations are presented for the selection indexes of the experiments previously carried out by Sumner and by Dice of the proportion of conspicuously colored prey taken by predators. In two of these experiments the indexes of selection against the conspicuously colored animals are $-.09$ and .13, respectively, which are insignificant deviations from the 1 : 1 ratio expected in the absence of selection. The other ten experiments

give selection indexes ranging from .22 to .57, all of these being highly significant.

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NOTES AND COMMENT

MATING BETWEEN TWO STRAINS OF DROSOPHILA MELANOGASTER

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Received June 2, 1949

Considerable study has been made of mating in the genus Drosophila, but many aspects of the process are not yet well understood. Much of the recent work (e.g. Dobzhansky, Mayr, etc.) has been designed to investigate the nature and origin of sexual isolation. Many sympatric but distinct species are not separated by sterility barriers, yet fail to cross because of sexual isolation. In such cases, if present theories of species origin are accepted, sexual isolation must have developed between similar groups such as geographic strains or races. Otherwise, when the two lines once again inhabited the same territory, any differences between them would be lost by interbreeding. For this reason, different strains or races of various species have been tested by investigators for indications of incipient sexual isolation.

The present work with Lausanne Special and Oregon R strains of *D. melanogaster* (Bridges and Brehme, 1944) was undertaken to test the possibility of sexual isolation between strains and to study the mating behavior of the flies. The results showed no indication of sexual isolation between the strains, unlike the unpublished results of Mather and Harrison, who found isolation between geographic races and also between selection lines of *D. melanogaster*. However, the data are of interest because of their bearing on the approach to the problem of sexual behavior and sexual isolation in Drosophila.

Each strain of flies had at one time been inbred (brother x sister) for many generations, but this close inbreeding had been discontinued. However, there must have been a fairly high degree of genetic homogeneity within each strain. The flies were cultured, and the experiments conducted in a constantly lighted room at $20.0 \pm 0.5°$ C. Virgin males and females were aged separately prior to the mating tests for periods ranging from 48 hours to 14 days, the usual period being about a week. After aging, one male and one female were introduced into an empty glass vial without etherization. Four types of matings were possible, each of which was made up in triplicate. Twelve vials were thus under observation at once, and all types of matings were observed simultaneously in order to minimize unknown or chance environmental fluctuations. All flies used in any set of twelve vials were of the same age.

Three readings were taken: the time the flies were introduced into the vial, the time copulation began, and the time it was terminated. The span between the first two readings may be called the courtship period although the proportion of time spent in actual courtship was variable. The data are shown in tables I and II. If copulation did not occur within 60 minutes, the courtship was considered incomplete even though longer observation showed that a few matings did occur within the next hour. These few matings are included in table II for the duration of copulation but are listed as incomplete in table I.

The data show that there was a characteristic courtship period for each type of female no matter which male was present. On the other hand, although the difference is not so striking, the duration of copulation is a characteristic of the male type. Both differences are statistically highly significant.

TABLE I. *Duration of Courtship*

Mating	n	% Complete in 60 min.	$\bar{m}$ in min.	$\bar{m}$ for ♀ type
♀ LS × ♂ LS	56	57.2	25.22	24.42 ± 1.83
♀ LS × ♂ OR	56	62.5	23.69	
♀ OR × ♂ LS	57	75.5	11.54	10.50 ± 0.97
♀ OR × ♂ OR	58	82.8	9.58	

Difference between means = 13.92 ± 2.07

TABLE II. *Duration of Copulation*

Mating	n	$\bar{m}$ in min.	$\bar{m}$ for ♂ type
♀ LS × ♂ LS	36	23.47	23.18 ± 0.48
♀ OR × ♂ LS	44	22.93	
♀ LS × ♂ OR	35	20.69	21.00 ± 0.34
♀ OR × ♂ OR	48	21.23	

Difference between means = 2.18 ± 0.58

Discussion. It is difficult to evaluate the role of one of the sexes in mating because there is no certainty that the behavior of the partner is constant in every case. The study of mating is, therefore, the study of the interactions between two individuals. However, it is not necessarily true that the roles of the males and the females are of equal importance during all phases of mating. For example, if the female remains in one place while the male seeks her out, failure by the male to seek the female would prohibit mating regardless of the female's state of receptivity.

The data indicate that, in *D. melanogaster*, the length of the courtship period depends chiefly on the female, but that the male determines the duration of copulation. If the duration of copulation were not determined by the male, it might be terminated by the female prior to the completion of the delivery of the sperm. Since the males failing to complete copulation would leave fewer offspring, control of this phase of mating should, theoretically, have developed in the males during the course of evolution. In the *willistoni* group, Spieth (1948) observed a single mating between a *D. sucinea* male and a *D. nebulosa* female. He found that the duration of copulation was comparable to that characteristic of *sucinea*. It appears, therefore, that in interspecific as well as intraspecific crosses, the male determines the duration of copulation.

Even more interesting is the conclusive evidence that the length of the courtship period is a characteristic of the female type. The possible importance of female discrimination has been suggested by Bateman (1948), and by data of Streisinger (1948). Although, from mating to mating, there was considerable variation in the intensity of the male's courting and in the length of the courtship, it is clear that the female must determine when mating is to occur. Bateman noted that not only was fertility reduced in weak inbred stocks, but also that the first mating did not occur as soon. It is probable that the large difference in average courtship period between the two female types was due to a higher degree of inbreeding in the L-S stock. Whether due to differences in the amount of inbreeding or to other differences between the strains, the conclusion as to the major role of the female during courtship is the same. Other evidence (Streisinger, 1948; Merrell, in press) has shown that mating by the males is more nearly at random than by the females. Therefore, if the females determine the length of the courtship period and the males mate practically at random, the females must be primarily responsible for the origin of sexual isolation in Drosophila. Spieth (1949) has found that in interspecific matings the male, as well as the female, may break off the courtship. However, such behavior is found only in species already well isolated from one another. In more closely related groups showing some sexual isolation, the female seems to be chiefly responsible. In studies on the origin of sexual isolation, the method most widely used has been a multiple choice technique whereby one kind of males is placed in a container with two different kinds of females (Dobzhansky and Mayr, 1944). The randomness of mating is determined by examination of the seminal receptacles after the elapse of sufficient time for about half of the females to be inseminated. The technique may actually measure a difference in the length of courtship necessary for the different female types.

A most puzzling fact is that mature females in 30.4% of the cases did not mate within 60 minutes. These incomplete matings were never due to failure by the males to court the females. While some females mated with practically no courtship by the male, others "refused" to mate in spite of persistent and prolonged courtship. Even though mating probably would occur eventually in most of these cases, such variation must have some physiological basis.

Summary. No sexual isolation was found between the Lausanne Special and Oregon R strains of *D. melanogaster*. However, the females of each strain showed a characteristic difference in the length of the courtship period no matter which male was present. The duration of copulation differed according to the strain of the male.

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THE TWO "VARIETIES" OF *DROSOPHILA MONTIUM*

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The recent comments of Freire-Maia (1949) in this column entitled "Balanced polymorphism in *Drosophila montium*" should be of interest to present-day taxonomists as offering additional evidence of a simple genetic basis for the antiquated systematic term "variety." Modern Drosophila taxonomists seldom use the term, and subspecies are rarely designated without careful genetic study. It has not always been so. In the many descriptive papers of Dr. O. Duda, for example, a large number of varieties were erected, some of them having been considered earlier as distinct species. The modern Drosophila worker is at a loss to know how to interpret them.

Drosophila montium was first described by de Meijere in 1916 from Tjibodas of Java. In 1924, Dr. Duda described two varieties, *xanthopyga* and *atropyga*, from Java and Formosa (but not *respectively* as indicated by Kikkawa and Peng, 1938). The former was distinguished from the latter by the fact that the last abdominal tergite was yellow in color instead of black as in the latter. This color difference is undoubtedly that found by Freire-Maia in Brazil and which was shown to be due mainly to a single autosomal gene. It seems logical to suggest that future use of the terms *"xanthopyga"* and *"atropyga"* should be discontinued since they most likely represent only individual genetic phenotypes and further, as individual variants rather than populations, are not deserving of taxonomic designation.

One might wish to suppress all varietal names appearing in the older taxonomic literature but, unfortunately, the varieties of former taxonomists do not always have such a simple genetic explanation. Such a situation is demonstrated by Duda's (1925) designation of *D. fasciola, pygmaea, prorepleta, ramsdeni, hydei* and *mulleri* as varieties of *D. repleta*, whereas laboratory tests have shown conclusively that at least the last two forms must be considered as distinct species.

Little is known concerning the geographic distribution of the two color phases of *D. montium*. Freire-Maia states that in natural populations of southwestern Brazil both dark and light individuals are found. The extensive collections reported by Kikkawa and Peng (*op. cit.*) for the Japanese Islands, however, revealed only specimens belonging to the light form. Living stocks maintained by the Texas laboratory from Japan, Hangchow, China and from Oahu, Hawaii similarly contain only light-colored individuals. Sturtevant (1927) listed only *"atropyga"* from India but the reference was based upon a single male specimen.

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